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Ontogenic Corticosteroidogenesis of the Domestic Fowl: Response of Isolated Adrenocortical Cells[1] (42498)

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Abstract. Ontogenic adrenocortical function of the domestic was investigated using adrenocortical cells isolated from embryonic chicks (18, 19, 20, and 21 days old) and male and female posthatch birds (1 day, 1 week, and 3 weeks old). Production of the predominant corticosteroids secreted by the chicken adrenal gland, corticosterone, cortisol, and aldosterone, was measured by radioimmunoassay after 2-hr incubation of cells with or without steroidogenic agents. Approaching hatch, basal and maximal ACTH-(1-24) (ACTH)-induced corticosteroid production increased steadily and peaked around 1 day posthatch (5–18 times and 3–9 times, respectively, the production values at 18 days embryonic life). Thereafter, corticosteroid production values decreased steadily to 3 weeks posthatch. Corticosterone predominated over the ages studied: Maximal ACTH-induced corticosterone production averaged 52 and 115 times the production values of aldosterone and cortisol, respectively. In addition, maximal ACTH-induced aldosterone production was roughly 2.2 times greater than cortisol production over the ages studied except for a short-lived, disproportionately greater aldosterone production at 1 day posthatch. In addition to perihatch and age-related differences in cellular corticosteroid production, there were also differences in cellular sensitivity to steroidogenic agents as indicated by the differences in half-maximal steroidogenic concentration values (ED_{50} values) of the steroidogenic agents. Sensitivity to ACTH increased 2.7 times from Day 18 of embryonic life to 1 day posthatch and then decreased steadily to 3 weeks posthatch. In addition, sensitivity to 8-bromo-cAMP (8-Br-cAMP) increased abruptly at 1 day posthatch (nearly 3 times) but then remained constant thereafter. However, a consistent change in cellular sensitivity to 25-hydroxycholesterol was not observed until 3 weeks posthatch (an increase in sensitivity of 3 times that at Day 18 of embryonic life). These data of cellular sensitivity suggest that there were distinct developmental and maturational alterations in the cellular loci at which ACTH, 8-Br-cAMP, and 25-hydroxycholesterol acted. Thus, during the transition from embryonic to postembryonic life of the domestic fowl, there are alterations in adrenocortical cell steroidogenic capacity and in the function of some cellular loci comprising the corticosteroidogenic pathway.

In several mammalian species, as for example, the rat (1, 2), rabbit (2, 3), guinea pig (4), sheep (5, 6), monkey (7), and human (8), there is a recrudescence of adrenocortical activity near term. The subsequent increase in fetal plasma corticosteroids is a requirement for the maturation of many fetal organ systems (9) and is instrumental in the initiation of parturition in ruminants (10). Several studies *in vivo* and *in vitro* indicate that alterations in adrenocortical function per se are, in part, responsible for the marked increase in fetal plasma corticosteroids before birth. Near term there is evidence for an increase in adrenal response to ACTH (11), an increase in adrenal sensitivity to ACTH (5, 6, 12), and an increase in adrenal ACTH receptors (6). Studies *in vitro* using isolated adrenocortical cells have been especially important in understanding the cellular mechanisms underlying the alterations in adrenocortical function near term (6, 13–15).

In contrast to what is known about mammalian adrenocortical function during the perinatal period, less is known about avian adrenal function during the perihatch period. In the domestic fowl, there is evidence for an increase in adrenal glucocorticoid content (16–

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19), and an increase in allantoic (20) and plasma (17, 19–21) glucocorticoid concentrations approaching hatch. However, there is little information on the dynamics of adrenocortical function per se during the perihatch period (22–24). In addition, prior to this report, there have been no reports on the characterization of avian adrenocortical cell function during the transition from embryonic to postembryonic life.

Accordingly, in the present study we measured the influence of steroidogenic agents on enriched adrenocortical cell populations isolated from domestic fowl during the perihatch period. We evaluated some basic parameters of adrenocortical function, namely, basal and maximally stimulable production of the predominant and physiologically important chicken corticosteroids, corticosterone, cortisol, and aldosterone, and adrenocortical cell sensitivity to steroidogenic agents. We present evidence indicating that approaching hatch there are marked increases in adrenocortical cell steroidogenic capacity, and cellular responsiveness and sensitivity to ACTH; after hatch these increases in cellular steroidogenic capacity and sensitivity to ACTH decline gradually.

Materials and Methods. *Animals.* Male and female White Leghorn domestic fowl were used in this study. Males and females were pooled in experiments using 18-, 19-, 20-, and 21-day-old embryonic chicks, whereas sexes were kept separate in experiments using 1-day-, 1-week-, and 3-week-old birds. Fertilized eggs were collected on separate days and maintained at room temperature until a suitable number was achieved. Then eggs were incubated simultaneously to provide birds having roughly synchronous development and time of hatch. Posthatch birds were housed in Petersime brood units under a 16-hr light, 8-hr dark photocycle and had free access to a standard commercial diet, water, and heat. At each age, all animals were handled together under identical conditions to normalize stressing factors. The numbers of birds used in each of three experiments for each age were 40 for each embryonic age, 30 for each sex of 1-day-old posthatch birds, and 25 for each sex of 1-week- and 3-week-old posthatch birds. These numbers of birds reflect the amount of adrenal gland tissue required to yield a sufficient quantity of cells to test cell responses to steroidogenic agents and to achieve equal cell concentrations for each age studied. All animals were killed by decapitation and exsanguination. The adrenal glands were quickly removed, trimmed free of adhering connective tissue, and diced into small pieces for cell isolation.

Cell isolation and incubation. The standard medium for cell isolation and incubation was Krebs–Ringer Hepes buffer (24.2 m*M* Hepes, 118.5 m*M* NaCl, 4.75 m*M* KCl, 2.54 m*M* $CaCl_2$, 1.20 m*M* KH_2PO_4 1.20 m*M* $MgSO_4$, and 11.1 m*M* glucose, pH 7.5).

Adrenocortical cells were isolated and partially purified as described in detail previously (25). In brief, diced adrenal glands were treated with the standard medium containing collagenase (0.2%, Type I; Sigma Chemical Co., St. Louis, MO) and mechanical agitation until tissue fragments were completely dissociated. Adrenocortical cells were partially purified by using Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) continuous density gradient (40%) centrifugation. Routinely 85–89% purity was achieved by this method.

Enriched adrenocortical cells were suspended in the standard medium containing 0.25% BSA (Fraction V; Sigma Chemical Co.). Aliquots of cell suspensions of equal cell concentrations were incubated with various concentrations of ACTH-(1-24) (ACTH) (Cortrosyn, Organon, Inc., West Orange, NJ), 8-bromo-cyclic AMP (8-Br-cAMP; Sigma Chemical Co.) and 25-hydroxycholesterol (Steraloids Inc., Wilton, NH). Prior to the addition of these agents to the cell suspensions, ACTH was dissolved in a solution of 0.9% NaCl, pH adjusted to 3.0 with 1.0 *N* HCl, containing 0.1% BSA, whereas 8-Br-cAMP and 25-hydroxycholesterol were made up in the incubation medium. Initial dilutions of 25-hydroxycholesterol were carried out in absolute ethanol. However, the final ethanol concentration in the incubation did not exceed 1%; this concentration of ethanol did not affect corticosteroidogenesis. The incubation volumes were 200–300 μl (90% of the incubation volume was cell suspension, 10% was a solution containing a steroidogenic agent). Cells plus agents were incubated for 2 hr at 39.5–

41.0°C. In each experiment, at least 87% of the cells were viable after incubation as indicated by trypan blue dye exclusion.

Radioimmunoassay for corticosteroids. Cell incubations were assayed directly for the major chicken end product corticosteroids, corticosterone, cortisol, and aldosterone. Corticosterone, the predominant end product corticosteroid secreted by the domestic fowl adrenal gland (18, 19, 26), was measured by a modification of the radioimmunoassay procedure of Roy *et al.* (27) using specific antibody (Miles Research Products, Elkhart, IN). Cortisol and aldosterone were measured by using highly specific radioimmunoassay kits (antibody-coated tubes, ^{125}I-corticosteroids; Diagnostic Products, Inc., Los Angeles, CA). Corticosterone and cortisol crossreactivities in the aldosterone radioimmunoassay were 0.002% and below detection, respectively; aldosterone and corticosterone cross-reactivities in the cortisol radioimmunoassay were 0.01 and 0.4%, respectively. Radioimmunoassay of aliquots of the same pooled cell incubations showed intraassay and interassay variances of 7.6 and 10.7%, respectively.

Analysis of data. The responses of isolated adrenocortical cells from birds of various ages were determined. The average within- and between-experiment variances of cell incubations (calculated from basal corticosteroid values after 2 hr of incubation) were 11.3 and 17.5%, respectively. Data comprising the sigmoidal dose–response curves shown in this report were analyzed using a four-parameter logistic equation model (28) that is available as a computer program (ALLFIT, Biomedical Computing Technology Information Center, Vanderbilt Medical Center, Nashville, TN). This program allowed the simultaneous testing and statistical analysis of a family of sigmoidal dose–response curves. Steroidogenic agent ED_{50} values and maximal corticosteroid production values were calculated and statistically analyzed using this program.

Other data were statistically analyzed by analysis of variance (29). Data are expressed as the means ± SEM; means were deemed significantly different at $P \leq 0.05$.

Results. *Basal corticosteroid production by isolated adrenocortical cells from embryonic and postembryonic domestic fowl.* Regardless of age, corticosterone was the predominant corticosteroid basally produced by isolated cells (2.5×10^5 cells/ml; Fig. 1); on the average, basal (2 hr) corticosterone production was 8 and 48 times greater than basal aldosterone and cortisol production, respectively. In addition, basal aldosterone production was roughly 6 times greater than basal cortisol production. However, corticosteroids followed similar patterns of basal production over the ages studied. There was an abrupt rise in corticosteroid production approaching hatch, peaking between hatch day and 1 day posthatch (5–18 times the basal production values at 18 days embryonic life). Thereafter, basal corticosterone and aldosterone production declined steadily to 3 weeks posthatch, whereas basal cortisol production fluctuated.

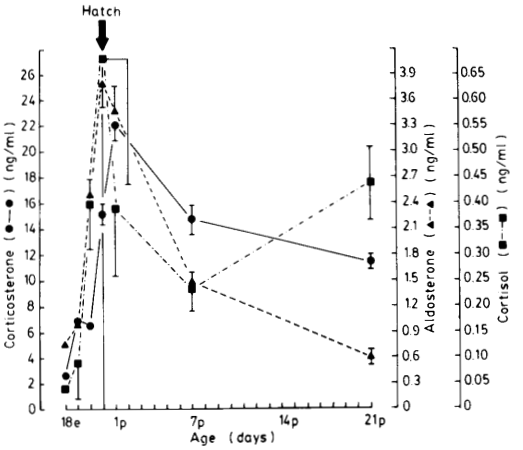

FIG. 1. Basal corticosteroid production by isolated adrenocortical cells from embryonic and posthatch domestic fowl. Embryonic ages are denoted with the letter *e* and posthatch ages are denoted with the letter *p*. Adrenocortical cells (2.5×10^5 cells/ml) were incubated for 2 hr. Adrenal glands from male and female embryonic chicks were pooled for cell isolation. In addition, data were pooled from experiments with cells from male and female posthatch birds. Each symbol represents the mean of corticosteroid values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

Maximal ACTH-induced corticosteroid production by isolated adrenocortical cells from embryonic and postembryonic domestic fowl. As seen with basal corticosteroid production, corticosterone was the predominant cortico-

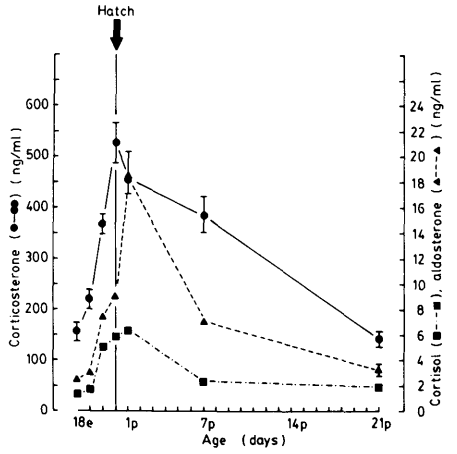

FIG. 2. Maximal ACTH-induced corticosteroid production by isolated adrenocortical cells from embryonic and posthatch domestic fowl. Embryonic ages are denoted with the letter *e* and posthatch ages are denoted with the letter *p*. Adrenocortical cells (2.5×10^5 cells/ml) were incubated with a maximal steroidogenic concentration of ACTH (1×10^{-9} *M*) for 2 hr. Adrenal glands from male and female embryonic chicks were pooled for cell isolation. In addition, data were pooled from experiments with cells from male and female posthatch birds. Each symbol represents the mean of corticosteroid values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols.

steroid produced by isolated cells (2.5×10^5 cells/ml) maximally stimulated by ACTH (1×10^{-9} *M*) for 2 hr (Fig. 2). On the average, maximal corticosterone production was 52 and 115 times greater than maximal aldosterone and cortisol production, respectively, and maximal aldosterone production was roughly 2.2 times greater than maximal cortisol production. Here again, corticosteroids followed similar patterns of maximal production over the ages studied. There was an abrupt rise in maximal corticosteroid production approaching hatch, which peaked between hatch day and 1 day posthatch (3–9 times the maximal production values at 18 days embryonic life). Thereafter, maximal corticosteroid production declined to 3 weeks posthatch.

Other differences in maximal corticosteroid production with age were apparent when the ratios of cortisol to corticosterone and aldosterone to corticosterone values were examined (Fig. 3). Differences in these ratios remained fairly constant over the ages studied with the exception of 1 day posthatch where there was a disproportionately greater production of aldosterone compared to cortisol (Figs. 2 and 3).

Response of isolated adrenocortical cells to various concentrations of ACTH, 8-Br-cAMP, and 25-hydroxycholesterol. In the aforementioned experiments, a high concentration of adrenocortical cells (2.5×10^5 cells/ml) was used to achieve sufficient corticosteroid production in order to detect aldosterone and cortisol. In addition, male and female adrenal glands were pooled for the isolation of adrenocortical cells. In the following experiments a lower concentration of adrenocortical cells (6.0×10^4 cells/ml) was used to investigate cellular responses to a wide range of concentrations of ACTH, 8-Br-cAMP, and 25-hydroxycholesterol. In these experiments, male and female adrenal glands were pooled for embryonic (*in ovo*) ages, whereas adrenal glands from the sexes were kept separate for

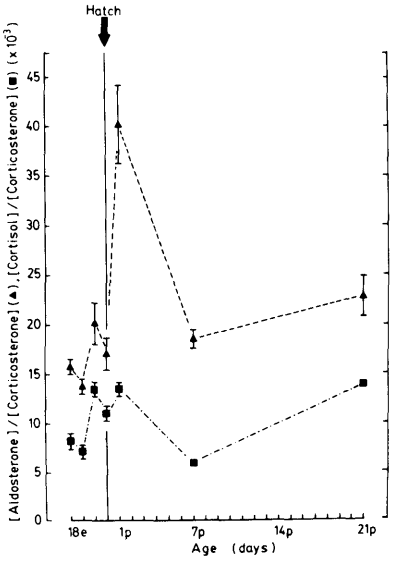

FIG. 3. Maximal ACTH-induced cortisol and aldosterone production expressed as a ratio of maximal ACTH-induced corticosterone production. Embryonic ages are denoted with the letter *e* and posthatch ages are denoted with the letter *p*. Each symbol represents the mean of quotient values of the data shown in Fig. 2. SEM are represented by bars when they are larger than the symbols.

posthatch ages. Corticosterone, the predominant corticosteroid produced by adrenocortical cells regardless of fowl age, served as the indicator of cellular response. The results are shown in Figs. 4–6.

Steroidogenic agents stimulated and supported cellular corticosterone production in a dose-dependent manner over approximately two log orders of concentration, regardless of fowl age or sex. To evaluate cellular responses, the dose–response curves were analyzed by computer (see Materials and Methods). Maximal corticosterone production values and steroidogenic agent ED_{50} values were calcu-

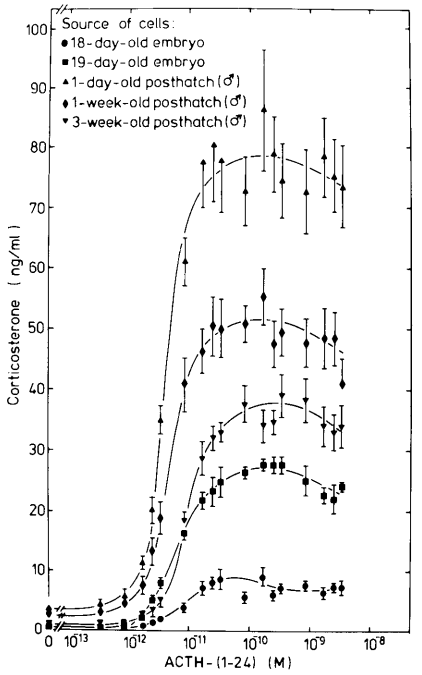

FIG. 4. ACTH-induced corticosterone production by isolated adrenocortical cells from embryonic and posthatch domestic fowl. Adrenocortical cells (6.0×10^4 cells/ml) were incubated with various concentrations of ACTH for 2 hr. Adrenal glands from male and female embryonic chicks were pooled for cell isolation, whereas adrenal glands from male and female posthatch birds were kept separate for cell isolation. Each symbol represents the mean of corticosterone values from nine cell incubations (three cell incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols. Only data from experiments with cells from male posthatch birds are shown. Similar results were obtained with female posthatch bird cells (see Tables I and II).

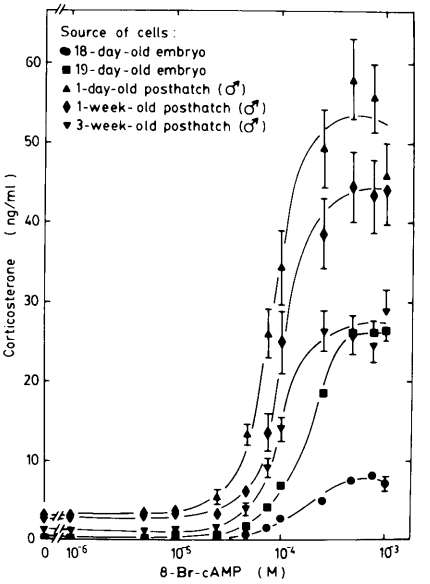

FIG. 5. 8-Br-cAMP-induced corticosterone production by isolated adrenocortical cells from embryonic and posthatch domestic fowl. Adrenocortical cells (6.0×10^4 cells/ml) were incubated with various concentrations of 8-Br-cAMP for 2 hr. Adrenal glands from male and female embryonic chicks were pooled for cell isolation, whereas adrenal glands from male and female posthatch bird were kept separate for cell isolation. Each symbol represents the mean of corticosterone values from nine cell incubations (three incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols. Only data from experiments with cells from male posthatch birds are shown. Similar results were obtained with female posthatch bird cells (see Tables I and II).

lated (Tables I and II). The data show clearly that maximal corticosterone production abruptly peaked by 1 day posthatch and then gradually and steadily declined to 3 weeks posthatch (Table I). Regardless of steroidogenic agent, maximal corticosterone production of cells from 1-day-old posthatch birds was roughly 6–11 times that of cells from 18-day-old embryos. In addition, basal cellular corticosterone production followed a pattern similar to maximal corticosterone production over the ages studied (Table I). These data corroborate the data from the previous experiments in which a high concentration of cells (2.5×10^5 cells/ml) and a fixed, maximally steroidogenic concentration of ACTH (1×10^{-9} *M*) were used (Fig. 2).

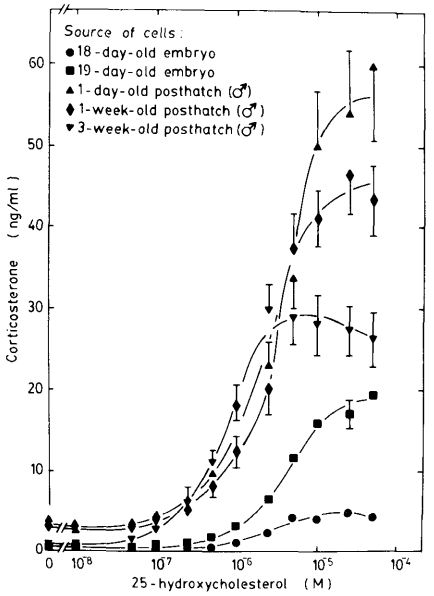

FIG. 6. Corticosterone production by isolated adrenocortical cells from embryonic and posthatch domestic fowl supported with 25-hydroxycholesterol. Adrenocortical cells (6.0×10^4 cells/ml) were incubated with various concentrations of 25-hydroxycholesterol for 2 hr. Adrenal glands from male and female embryonic chicks were pooled for cell isolation, whereas adrenal glands from male and female posthatch birds were kept separate for cell isolation. Each symbol represents the mean of corticosterone values from nine cell incubations (three incubations from each of three experiments). SEM are represented by bars when they are larger than the symbols. Only data from experiments with cells from male posthatch birds are shown. Similar results were obtained with female posthatch birds cells (see Tables I and II).

Other data suggest that cellular sensitivity to steroidogenic agents changed over the ages studied. This is evident from the changes in the ED_{50} values for steroidogenic agents (the lower the ED_{50} value the greater the cellular sensitivity (30); Table II). An index of cellular sensitivity can be obtained by calculating the reciprocal of the ratio of a steroidogenic agent ED_{50} value for cells from birds of any age to the ED_{50} value for cells from 18-day-old embryos. For example, ACTH ED_{50} values decreased abruptly to a minimal value at 1 day posthatch; thus, the sensitivity of cells from 1-day-old posthatch birds to ACTH was 2.7 times that of cells from 18-day-old embryos. Thereafter, sensitivity to ACTH declined such that the sensitivity of cells from 3-week-old posthatch fowl was the same as that of cells from 18-day-old embryos. There were also differences in cellular sensitivity to 8-Br-cAMP and 25-hydroxycholesterol over the ages studied. However, the changes in cellular sensitivity to these agents were distinct from those to ACTH: Cellular sensitivity to 8-Br-cAMP increased nearly 3 times by 1 day posthatch, but then remained constant through 3 weeks posthatch, whereas a consistent increase (3.2 times) in cellular sensitivity to 25-hydroxycholesterol was not apparent until 3 weeks posthatch.

Discussion. The present study suggests that the steroidogenic capacity of domestic fowl adrenocortical cells changed with the transition from embryonic to postembryonic life. On an equal cell concentration basis, approaching hatch day there was a rapid increase in basal and maximal ACTH-stimulable corticosteroid production. Thereafter (with the exception of basal cortisol production), there was a gradual decline in corticosteroid production to 3 weeks posthatch (Figs. 1 and 2). Our data are consistent with reports of an increase in adrenal glucocorticoid content (16–19) and increases in allantoic (20) and plasma (17, 19–21) glucocorticoid concentrations approaching hatch, and the subsequent decline in plasma glucocorticoid concentrations after hatch (18, 19, 21, 22, 31). Moreover, our data indicate that changes in adrenocortical cell function per se, in part, are responsible for these alterations in corticosteroid parameters with domestic fowl development and posthatch maturation.

The differences in steroidogenic capacity over the ages studied appear to be due to differences in the amount of active steroidogenic enzymes within the cells. At each age studied, maximal corticosterone production values of cells supported by the precursor, 25-hydroxycholesterol, and of cells stimulated by ACTH and 8-Br-cAMP were similar (Figs. 4–6 and Table I). However, whether these changes in steroidogenic capacity were due to age-related alterations in the recruitment of active steroidogenic enzymes from an inactive pool or due to alterations in *de novo* synthesis and inactivation/degradation of enzymes is unclear. An equally plausible explanation is the diversion of the steroidogenic pathway from the

TABLE I. BASAL AND MAXIMAL CORTICOSTERONE PRODUCTION (ng/ml) BY EMBRYONIC AND POSTHATCH DOMESTIC FOWL ADRENOCORTICAL CELLS

Source of cells	Basal	ACTH	8-Br-cAMP	25-hydroxycholesterol
18-day-old embryo (♂ + ♀)	0.13 ± 0.02	9.10 ± 0.89	9.21 ± 1.36	7.02 ± 1.22
19-day-old embryo (♂ + ♀)	0.52 ± 0.01[a]	26.97 ± 0.59[a]	27.30 ± 0.50[a]	18.76 ± 1.13[a]
1-day-old posthatch (♂)	3.27 ± 0.22[a]	77.21 ± 0.42[a]	53.92 ± 0.50[a]	58.62 ± 1.27[a]
1-day-old posthatch (♀)	3.62 ± 0.33[a]	49.55 ± 0.43[a]	48.11 ± 0.35[a]	61.08 ± 0.53[a]
1-week-old posthatch (♂)	2.83 ± 0.12[a]	50.76 ± 0.45[a,b]	43.50 ± 0.35[a,b]	46.09 ± 0.93[a,b]
1-week-old posthatch (♀)	1.96 ± 0.08[a,b]	41.59 ± 0.54[a,b]	44.26 ± 0.33[a,b]	48.42 ± 0.76[a,b]
3-week-old posthatch (♂)	1.15 ± 0.05[a,b]	37.39 ± 0.61[a,b]	27.30 ± 0.40[a,b]	31.72 ± 2.04[a,b]
3-week-old posthatch (♀)	0.85 ± 0.02[a,b]	32.05 ± 0.58[a,b]	27.27 ± 0.32[a,b]	33.50 ± 0.62[a,b]

Note. Domestic fowl adrenocortical cells (6.0×10^4 cells/ml) were incubated with or without various concentrations of ACTH or 8-Br-cAMP or 25-hydroxycholesterol for 2 hr. The dose–response data (Figs. 4–6) were statistically analyzed using the ALLFIT computer program (see Materials and Methods). Each value is the calculated mean ± SEM (ALLFIT) from the data of three experiments.

[a] Significantly different from value for 18-day-old embryo ($P \leq 0.05$).

[b] Significantly different from value for 1-day-old posthatch of corresponding sex ($P \leq 0.05$).

production of undetected steroids to the production of detectable physiologically important corticosteroids, corticosterone, cortisol, and aldosterone. Indeed, Gonzalez *et al.* (23) have demonstrated that adrenal 5β-reductase greatly competes with enzymes of the corticosterone pathway for progesterone and that this competition decreases precipitously from Day 17 of embryonic life to hatch.

Other work suggests that adrenal cortisol secretion declines to undetectable levels during the transition from embryonic to postembryonic life (18, 22, 32). The present study does not support the previous work of others. Basal and maximal ACTH-induced cortisol production (Figs. 1 and 2) and the ratio of cortisol to corticosterone (Fig. 3) persisted to 3 weeks posthatch. Our results with isolated adrenocortical cells are consistent with reports of a rapid increase in corticosterone and cortisol concentrations in the domestic fowl (19) and duck adrenal (33) approaching hatch and the gradual decline in the concentrations of these corticosteroids after hatch (19, 33) but with the persistence of cortisol. Discrepancies between our work and the work of others (18, 22, 32) may be due to the fact that we used freshly isolated, intact adrenocortical cells in-

TABLE II. HALF-MAXIMAL STEROIDOGENIC CONCENTRATION (ED_{50}) OF STEROIDOGENIC AGENT

Source of cells	ACTH ($\times 10^{-12}$ *M*)	8-Br-cAMP ($\times 10^{-5}$ *M*)	25-hydroxycholesterol ($\times 10^{-6}$ *M*)
18-day-old embryo (♂ + ♀)	9.56 ± 2.11	20.77 ± 5.50	2.56 ± 1.16
19-day-old embryo (♂ + ♀)	6.53 ± 0.46[a]	17.19 ± 0.74	3.78 ± 0.49
1-day-old posthatch (♂)	4.10 ± 0.08[a]	8.30 ± 0.13[a]	3.25 ± 0.21
1-day-old posthatch (♀)	3.02 ± 0.07[a]	7.36 ± 0.11[a]	1.73 ± 0.06
1-week-old posthatch (♂)	4.71 ± 0.15[a,b]	9.71 ± 0.14[a,b]	2.72 ± 0.17
1-week-old posthatch (♀)	6.11 ± 0.27[a,b]	8.91 ± 0.12[a,b]	1.12 ± 0.04[a,b]
3-week-old posthatch (♂)	9.26 ± 0.43[b]	10.16 ± 0.30[a,b]	0.84 ± 0.12[a,b]
3-week-old posthatch (♀)	10.89 ± 0.49[b]	7.18 ± 0.15[a,b]	0.78 ± 0.04[a,b]

Note. Domestic fowl adrenocortical cells (6.0×10^4 cells/ml) were incubated with or without various concentrations of ACTH or 8-Br-cAMP or 25-hydroxycholesterol for 2 hr. The dose-response curves (Figs. 4–6) were statistically analyzed using the ALLFIT computer program (see Materials and Methods). Each value is the calculated mean ± SEM (ALLFIT) from the data of three experiments.

[a] Significantly different from value for 18-day-old embryo ($P \leq 0.05$).

[b] Significantly different from value for 1-day-old posthatch of corresponding sex ($P \leq 0.05$).

cubated briefly, whereas other investigators conducted studies *in vivo* (32) or *in vitro* using adrenal gland homogenates (18, 22). Moreover, even homogenates of adrenal glands from embryonic, immature, and mature domestic fowl produced cortisol when 17α-hydroxyprogesterone was used as a substrate (22), thus indicating that the potential to produce cortisol is present in the domestic fowl adrenal gland regardless of age.

Results of other work *in vivo* and *in vitro* (16, 18, 19, 21, 22, 32, 34) are equivocal in regard to the predominant glucocorticoid produced by the adrenal gland of the embryonic and posthatch domestic fowl. In the present study with isolated adrenocortical cells, corticosterone predominated over cortisol at the embryonic and postembryonic ages studied (Figs. 1 and 2). Our results with isolated adrenocortical cells are consistent with the results of work with adrenal tissue reported by Tanabe and co-workers (18, 19, 33). Furthermore, we show that aldosterone predominated over cortisol, thus indicating the predominance of the 21-hydroxylase pathway over the 17α-hydroxylase pathway. In addition, compared with maximal ACTH-induced cortisol production, there was a disproportionate increase in maximal ACTH-induced aldosterone production at 1 day posthatch (Figs. 2 and 3), thus suggesting an inexplicable short-lived increase in cellular 18-hydroxylase activity at that age.

Reports from studies *in vivo,* in which plasma corticosterone response to stress and exogenous ACTH were measured, suggest that there is a stress nonresponsive period in domestic fowl after hatch (24, 35, 36) and that this, in part, is due to a loss of adrenal responsivity to ACTH (24). For example, earlier *in vivo* work of Freeman and Flack (24) suggests that the adrenal gland of the 1-day-old chick is less responsive and less sensitive to ACTH than that of the 3-week-old bird. In contrast, our results suggest that the stress nonresponsive period in the domestic fowl is not due to a decrease in adrenocortical function per se: Cells from 1-day-old chicks had the greatest basal and maximal corticosterone production (Table I) and the greatest sensitivity to ACTH (as indicated by the lowest ED_{50} value; Table II) compared to cells from embryonic chicks and from older posthatch chickens. Furthermore, the recent work of Freeman and Manning (36) suggests that the stress nonresponsive period of the domestic fowl is due largely to a temporary inhibition of hypothalamic function.

There were also changes in cellular sensitivity to 8-Br-cAMP and 25-hydroxycholesterol, but they were distinct from those for ACTH in that they occurred at later ages and then remained constant. In support of the present study, previous work indicates that after 3 weeks of age, cellular sensitivity to ACTH continues to decline to sexual maturation, whereas cellular sensitivities to 8-Br-cAMP and pregnenolone remain constant (25). We hypothesize that the early transient increase in cellular sensitivity to ACTH (possibly including ACTH receptors) induced subsequent persistent increases in the sensitivities of intracellular loci (proteins, enzymes, etc.), to second messengers (e.g., cAMP), and to steroidogenic precursors (e.g., cholesterol and pregnenolone). However, we found perplexing the finding of persistent increases in cellular sensitivity to 8-Br-cAMP and 25-hydroxycholesterol in the presence of a continual age-related decline in cellular sensitivity to ACTH. Perhaps, as originally proposed by Frankel *et al.* (37), factors other than ACTH play an increasingly important role in stimulating corticosteroidogenesis during the posthatch life of domestic fowl. In support of this notion, previous work with domestic fowl indicates that adrenocortical cell steroidogenic capacity and sensitivity to ACTH are only partially reduced at as long as 24 days after hypophysectomy (38).

Results of the present work with adrenocortical cells from embryonic and postembryonic domestic fowl are consistent with the results of other work *in vitro* with mammalian adrenal tissue (3) and isolated adrenocortical cells (6, 13–15). Our work and the work of others suggest that increases in adrenocortical/neonatal life may be of general occurrence among the vertebrates. Therefore, further work with the embryonic and posthatch domestic fowl might be of value for the general understanding of adrenocortical development at the cellular level. In addition studies with domestic fowl may have an advantage over studies with

mammals since studies *in ovo* are not encumbered with maternal and other interfering factors that are present in studies using mammalian *in utero* models.

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