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Embryonic Pituitary Adrenal Axis, Behavior Development and Domestication in Birds

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SYNOPSIS. ACTH and corticosterone exert opposite effects on the approach and imprinting behavior of newly hatched ducklings. Wild mallard and domesticated Pekin ducklings differ in the early posthatch period in both plasma corticosterone levels and approach/avoidance behaviors. Injection of Pekin duckling embryos with pituitary-adrenocortical hormones alters both later adrenal function and certain aspects of posthatch behavior. These birds have behavioral and hormonal characteristics which resemble those of wild mallards. The hypothesis that behavioral differences in wild and domesticated ducklings result from a higher level of pituitary adrenal function in the wild embryo is explored. Although adrenocortical function changes during domestication in many species, evidence that the hormonal changes mediate the concomitant changes in approach and avoidance behavior remains inconclusive. Factors which cause adrenal function and early behaviors to differ in wild and domesticated genotypes must be sought in the gene action during embryonic development. Since imprinting behavior is modulated by pituitary-adrenal hormones, any factor which affects post-hatch adrenal function may potentially affect imprinting. Later behavior development in the adult is strongly dependent on neonatal experiences; and, therefore, hormonal modulation of early imprinting behavior may constitute an important determinant of adult social behavior.

INTRODUCTION

Hormones synthesized in embryonic endocrine tissues or contributed by the mother via the placenta may influence the development of the brain. This principle was pioneered by Jeffrey Harris (1964) and W. C. Young (1961) as a result of experiments with sex steroids in neonatal rodents. There is a growing body of evidence that corticosteroids may also act during development to influence the servomechanism regulating their secretion in later life (Levine and Mullins, 1966; Krieger, 1972; Lorenz *et al.*, 1974; Martin, 1975). The mechanism or mechanisms involved in the "organizational action" of corticosteroids are not clear, but may well include effects on induction of brain receptors (Lisk, 1971), on developing neurotransmitter systems (Vernadakis, 1971; Parvez and Parvez, 1973; Costa *et al.*, 1974; Phillipson and Sandler, 1975), on myelination of developing neurons (Granich and Timiras, 1971), on gene expression (Schwartz, 1972; Earp, 1974), on neuronal DNA/RNA content (Howard, 1965; Howard and Benjamins, 1975) and on neuronal morphogenesis (Shapiro and Vukovich, 1970).

It seems reasonable and predictable that these diverse actions of corticosteroids and ACTH would be manifested in the behavior of the animal at a later stage. Surprisingly, little work exists on the behavioral aspects of early hormone action with the exception of sex steroid action. This is certainly in part due to the difficulty of defining and distinguishing indirect and direct effects. Also, unlike sex steroids, hormones of the hypothalamic-pituitary-adrenal (HPA) system have no distinct class of behaviors which they control. Rather they influence a wide variety of behaviors ranging from sexual excitement (Bertolini, 1969; Deviche, 1976) to conditioned avoidance behavior (DeWied, 1974). Clinicians have long realized that

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disorders of the HPA system such as Cushing's Disease and Addison's Disease, include behavioral alterations. Extended treatment with steroid anti-inflammatory agents also may produce mood changes (Myles and Daly, 1974). These were thought to be side effects of a general metabolic derangement induced by the hormonal imbalance. That these hormones may have direct effects on the nervous system and hence on behavior was not appreciated until more recently (DeWied, 1974; Brush and Froehlich, 1975 for critical review). ACTH and fragments of this molecule, *e.g.*, $ACTH_{4-10}$, inhibit extinction of conditioned avoidance responses. Other molecules of similar structure, *e.g.*, MSH, have similar effects as do the vasopressins, *e.g.*, arginine vasopressin, lysine vasopressin, etc. On the other hand, certain steroids including progesterone, pregnenolone and corticosterone, but not testosterone or estradiol, have the opposite effect, *i.e.*, they facilitate extinction of conditioned avoidance behavior (DeWied, 1974). Kastin and co-workers have reported similar effects in rodents and additional effects on human performance, related to attention or motivation (Miller *et al.*, 1974; Kastin *et al.*, 1975). It is now clear that the HPA system modulates fear motivated behavior in rats (DeWied, 1966, 1974; Weiss *et al.*, 1970), but less clear whether developmental manipulation of this system also effects later fear-motivated behaviors (Swanson and McKeag, 1969; Martin, 1973, 1975; Van der Helm-Hylkema, 1973; Lat and Jakoubek, 1974). Before reviewing in more detail developmental studies on pituitary adrenocortical modulation of approach/avoidance behavior in birds, let us first examine three methodological points bearing on the study of hormone-behavior interactions.

METHODOLOGICAL PERSPECTIVES

Ethologists have long criticized studies of behavior which concentrate on isolated components of behavior without reference to the adapted whole because such studies may not provide useful generalizations and because they often overlook the true functional significance of patterns or effects. It is desirable to design behavioral tests which utilize the animal's natural capacities and proclivities. The behavior tasks used in many investigations of HPA function and behavior are tasks which the animal's evolution has not "anticipated" and for which no specific adapted response may exist. Studies of conditioned avoidance behavior fit clearly into this category. Endocrine responses to such tasks may represent random, inappropriate or even pathological actions or they may represent generalized coping responses which have evolved for other natural contingencies. Lacking has been a convincing demonstration that these hormones are involved in behavior under natural conditions. The studies reviewed below concern imprinting and innate avoidance behavior in waterfowl which are behaviors adapted for specific functions in the species' evolution.

The second methodological point concerns the use of birds as models for studying developmental hormone-behavior relationships. Manipulation of hormone levels in mammalian embryos presents technical problems since the placenta translocates and/or produces certain hormones and not others, and since the mother may metabolize drugs and hormones before they reach the embryo, etc. These problems are avoided when using birds since the egg albumin and yolk are slowly absorbed by the developing avian embryo and may hence serve as a reservoir for introducing hormones or other substances. Furthermore, certain birds, *e.g.*, ducklings, chicks, are precocial and begin responding to external stimuli within 12 hours of hatching. This reduces learning or experience as an important contributor to the variability of a behavioral response.

The third point concerns the use of different genotypes to elucidate hormone-behavior interactions. Closely related genotypes differing in a given behavior may show correlated hormonal differences. During domestication man has produced sub-species that are more docile than the wild type. Most domesticated ducks are derived from the mallard, *Anas platyrhynchos*, and they still interbreed with

the mallard readily. A study of their hormonal and behavioral differences may provide clues for unraveling the relationship between HPA function and approach/avoidance behaviors.

DOMESTICATION

Darwin once pointed out that domestication is a useful model of evolution. Artificial selection for docile sociable animals has produced striking parallel changes in a variety of species in their avoidance behavior.

Behavioral changes in the mallard

The domesticated duck differs from its wild counterpart, the mallard, in the rate of habituation to fear-provoking stimuli (Martin, 1973; Desforges and Wood-Gush, 1975). Martin (1973) devised an innate avoidance habituation test; this test consists of thrusting a puppet-like stimulus supported on a 1 m pole into the home cage of the duckling and recording its response on a scale devised by Schaller and Emlen (1962) (0 = no response; 6 = maximal). When given 4 trials every 15 min, twice daily for 5 to 10 days, the habituation curves indicate that the domesticated Pekin habituates more rapidly than the mallard (Fig. 1). This is unlikely to represent a simple strain difference unrelated to domestication because Desforges and Wood-Gush (1975) have shown a parallel effect in habituation of the domesticated Aylesbury Duck. These findings are of interest here for two reasons. Embryonic treatment of ducklings, but not acute treatment (Figs. 1 and 3), with ACTH decreases the habituation rate in this paradigm; and secondly, ACTH and ACTH analogues retard the extinction of conditioned avoidance behavior in rats (DeWied, 1966, 1974). Before discussing these points further, I will delineate some other ways in which wild and domesticated ducks differ.

The wild mallard also differs from the domesticated Pekin in its imprinting behavior. Imprinting is the process by which precocial bird species develop social attachments shortly after they hatch (Lorenz, 1935; Bateson, 1966; Hess, 1973). It involves a tendency to approach a moving object during a sensitive period after hatching and a process of learning or imprinting to the characteristics of that object. the Pekin duckling approaches a moving imprinting model in the first day after hatching more readily than its wild counterpart (Gottlieb, 1961). Moreover, a greater proportion of Pekin ducklings than mallard ducklings are attracted to a moving imprinting model. It seems likely that the imprinting "sensitive period" is broader and less sharply defined in the Pekin, and hence it may develop social attachments to a wider variety of objects. This may correspondingly reduce the range of objects it will later avoid. Any hormonal factors which limit or restrict the sensitive period will, therefore, reduce the class of objects for which the animal will develop a subsequent preference.

After only about 20 generations of captivity the mallard becomes "semi-domesticated" and ducklings become nota-

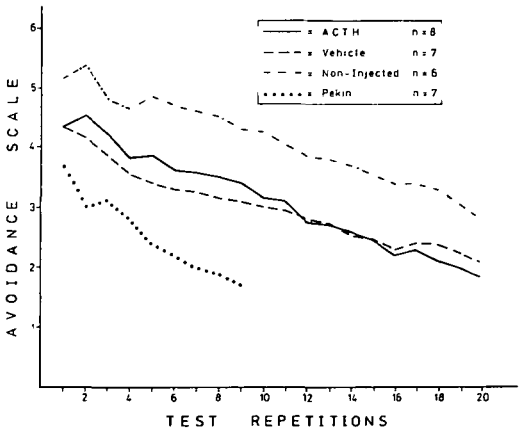

FIG. 1. Habituation of an innate avoidance response in Pekin ducklings and in mallard ducklings treated acutely with ACTH. Mallards treated 1) with 1 IU ACTH (Ferring) daily one hour before first avoidance test, 2) with carboxymethylcellulose vehicle or 3) untreated; Pekins were untreated. Avoidance test given two times daily; points represent group means for each test. Avoidance scale 0 = no response, 6 = maximal reaction to stimulus. Slopes for tests one through nine are: untreated mallards −.1069; ACTH-treated mallards, −.1407; vehicle-treated mallards, −.1461; untreated Pekins, −.2401. (Martin, unpublished data)

bly less responsive to fear-provoking stimuli such as a hawk shadow (Martin, 1971, 1975). Since strength of approach responses are generally inversely related to intensity of avoidance responses (Hess, 1959), changes in imprinting behavior also may result from captive selection. The genes responsible for mediating these differences in behavior presumably have their effect during embryonic development because the differences are apparent shortly after hatching. Since the changes in phenotype are apparent in a relatively short number of generations, relatively few genetic changes must be involved. Can this genetic change involve control of hormones of the pituitary-adrenal axis?

Changes in adrenal physiology in domesticated animals

C. P. Richter (1954) was the first to draw attention to the differences between the adrenal cortex of wild and domesticated rats. Wild rats have larger adrenals which are less easily depleted of ascorbic acid under stress (Richter, 1954). Further studies showed that the wild rat's adrenal contained larger cells and a more extensive capillary network in the *zona glomerulosa* and *zona reticularis* which pointed to an increased secretory capacity (Mosier, 1957). Similarly in the mouse three of four wild species, when compared to the inbred C57 B10 strain, exhibited a greater adrenocorticoid response to electric shock (Treiman and Levine, 1969). On the other hand, Belyaev and Trut (1975), in discussing their 15 year selection program at Novosibirsk to produce a domesticated fox for fur production, indicate that the line selected for docility usually exhibits a greater adrenocortical response to ACTH and to confinement stress, although the reverse occurred during February and March. Höhn *et al.* (1965) found a seasonal change in adrenal weights in the wild mallard which was positively correlated with the reproductive season; whereas, little seasonal change occurred in the Pekin. Mallards subjected to immobilization stress for 65 minutes exhibited a sustained elevation in plasma adrenocorticoids; whereas, the elevation in Pekin levels had returned to near normal by the end of the stress period (Martin, 1975). Table 1 illustrates the plasma adrenocorticoid response to jugular vein incision in three groups of 5 month old ducks, *Anas platyrhynchos*. The mallard group was hatched from eggs collected from a free living flock; the dwarf Pekin represents the domesticated strain, and the hybrid group was hatched from a single clutch resulting from mating a captive male mallard with a female dwarf Pekin. These data again suggest the mallard HPA axis is more responsive to stressors than that of the domesticated strains. However, it would be unwise at this time to conclude that domestication, *per se*, reduces pituitary adrenocortical responsiveness. The data presented by Belyaev and Trut (1975) for the silver fox do not conform to this pattern. Basal levels of plasma corticoids in semi-domesticated foxes were lower than those in the wild strain at all times of the year, and the docile strain showed a greater percent elevation following ACTH injection or stressors. Their interpretation was that selection for docility leads to lower plasma corticosteroid levels and to an altered state of the HPA system. Since the opposite patterns were obtained in the duck, the hypothesis that

TABLE 1. *Plasma adrenocorticoid response to jugular vein incision in subadult wild, domesticated and hybrid ducks.*

Strain	Sample size	Basal level[a] (Time 0)	Stress level (Time 0+1 min)	Percent increase
Mallard	4	.95 ± .365	11.0 ± 4.44	1572 ± 659.9
Hybrid[b]	5	5.72 ± 1.79	19.7 ± 3.35	298 ± 59.92
Dwarf Pekin	6	5.86 ± 2.29	10.9 ± 2.18	240 ± 159.9

[a] Plasma adrenocorticoid level in ng/ml ± S.E.M.
[b] Consists of sibling progeny of mallard male × Dwarf Pekin female.

domestication is accompanied by a reduction in the activity of the HPA system appears too general to be valid. More data from these and other species is clearly needed, and more specific hypotheses are required. For example, no information is available on the amount of unbound plasma corticosteroids in these strains or on the characteristics of the plasma binding protein, transcortin. Without this information and information about target tissue sensitivity, the significance of different hormone levels in domesticated and wild strains will remain unclear.

One approach which I have attempted is the gene action approach of Ginsburg (1958, 1971) in which one traces the developmental onset of differences in HPA function and correlates this onset with emergence of behavioral differences. By simulating the gene action of one strain through experimental manipulation of hormone levels in a second strain, one may produce a phenocopy of the first strain. If the phenocopy is identical to the phenotype of the other strain in its behavior (true phenocopy), then one has presumably identified the mechanism responsible for the behavioral difference. Likewise, significant similarity between the phenocopy and the other phenotype implies that the endocrine factor in question is a contributing factor to the behavioral differences in the two strains. Now, what information suggests that activity of the HPA system during development differs between the wild and domesticated duck?

Mallard ducklings hatch with higher plasma corticosterone levels than Pekins (Martin, 1975). Figure 2 illustrates how the plasma corticosterone (B) levels change in the two strains in the early post-hatch period.[1] In the domesticated strain the level rises during the first day after hatching only to decline again during the third day; whereas, the mallard level is high at hatching and appears to fall during the first post-hatch day. The increase in the plasma corticosteroids in the Pekin has been confirmed by Weiss *et al.* (1977). One may speculate that the mallard levels were higher during prehatch development as well.

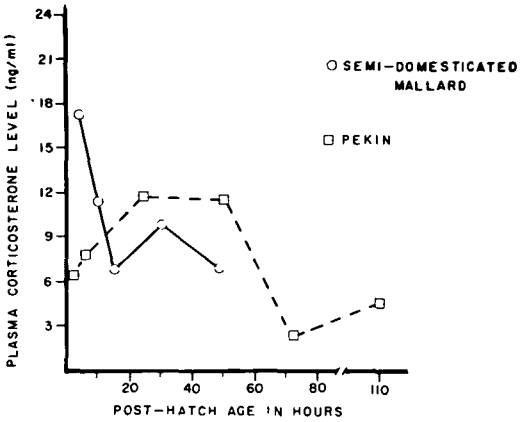

FIG. 2. Plasma adrenocorticoid levels following hatching in semi-domesticated mallard and domesticated Pekin ducklings. These mallards are termed semi-domesticated since the parental stock has been in captivity for more than 15 generations. Wild mallards have higher post-hatch levels (Martin, 1975). Points represent means of four to eight individuals, each measured in duplicate using CPB assay. Initial posthatch values differ between strains. $P < .05$, 2-tail, $U = 39.5$. Pekin level increases from 2 to 50 hr, $P < .01$, 2-tail, $U = 50$. Mallard regression equation from 2 to 50 hr is $y = 14.44 - .1575x$; slope may differ from 0, $F_{1,19} = 4.229$; $P < .10$. (Data based on Martin, 1975, and unpublished data)

EMBRYONIC ENDOCRINE MANIPULATION AND APPROACH-AVOIDANCE BEHAVIOR

In three separate experiments I attempted to change the level of HPA hormones in the developing Pekin duck embryo by injecting hormones or enzyme inhibitors. The egg injection procedure was adapted from the method of McLaughlin *et al.* (1963) using the diagrams of Romanoff (1960) as a guide. Zinc bound porcine ACTH (Schering) in doses of .005, .05, or .5 I.U. was injected four times during incubation. The first two injections given on days 14 and 18 were placed in the albumin or yolk; whereas, the last two, on days 22 and 26, were on the chorioallantoic membrane.

[1] No effort was made to separate the various glucocorticoids in the plasma; earlier workers have shown that corticosterone is by far the most abundant glucocorticoid in the duck (Bouille *et al.*, 1969) and in other avian species. New work by Kalliecharan and Hall (1974) suggests this assumption may not be valid for the developing chick embryo in which relatively large amounts of cortisol and cortisone were found.

Corticosterone was injected either prior to incubation (10μg) or during incubation on days 15, 19, and 23 (50 μg per injection). the 20α-hydroxylase inhibitor, aminoglutethimide (AG) (Elipten—Ciba Geigy), which blocks the conversion of cholesterol to pregnenolone, was administered as the phosphate salt on days 14, 18, 22, and 25 in doses of 1.25 mg/injection. This dose was effective in suppressing embryonic adrenal function in chicks (Adjovi and Idelman, 1969). The reader will find additional detail on the injection technique and procedure in Martin (1973).

In a fourth experiment I injected metyrapone (MET) (Metopirone—Ciba Geigy) into developing mallard eggs on days 20, 23, 26, and 27 in doses of .25, .25, .5 and 1 mg respectively. Metyrapone is an 11β-hydroxylase inhibitor which prevents the conversion of 11-deoxycorticosterone to corticosterone.

Certain aspects of these experiments are summarized in Figures 3 and 4. Summarized are the effects on imprinting behavior and avoidance habituation. The method of measuring imprinting behavior

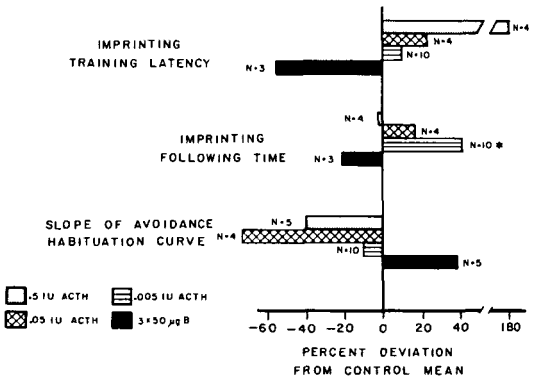

FIG. 3. Approach and avoidance behavior in Pekin ducklings treated during embryonic development with long-acting ACTH or with corticosterone. Following time with imprinting model in .005 IU ACTH group differs from vehicle-injected control and from .5 IU ACTH group (LSD = 135.5, $P < .05$; LSD = 144.2, $P < .05$, respectively). Combined ACTH groups differ from control ($P < .05$) in rate of habituation in innate avoidance test using parametric regression analysis and comparing the slopes of the habituation curves. Avoidance test given two times daily for four days. (Data based on Martin, 1975, and unpublished observations)

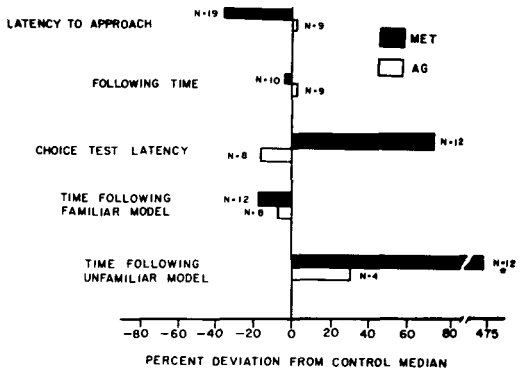

FIG. 4. Imprinting behavior in ducklings treated during embryonic development with inhibitors of steroid synthesis, metyrapone (MET) or aminoglutethimide (AG). MET causes learning impairment in mallard ducklings as measured by increased time following unfamiliar imprinting model in choice test ($U = 28, P < .025$). (Martin, unpublished data)

has already been described in detail (Martin and Schutz, 1975). Briefly, it consists of placing the 18-24 hr old duckling next to an imprinting model (red cube or blue ball) in a dark runway. The model begins to emit repetitive sounds from an internal loudspeaker; then the chamber is illuminated. Next, the vocalizing model begins to move away from the duckling. The experimenter measures the time taken by the duckling to begin following (Training Latency) and the time spent with the model (Following Time) during the 10 min training experience. Two days later the duckling is returned to the imprinting chamber with the familiar and an unfamiliar model present (Choice Test). The experimenter records the latency and time spent following each model. The training latency and following time are measurements of the animal's approach drive or, alternatively, of the degree of suppression of avoidance. The choice test provides a measure of how much learning occurred during the training period. Two aspects of the results from Fig. 3 deserve attention. First, embryonic injections of ACTH can alter imprinting behavior and avoidance habituation depending on the dosage, and secondly, ACTH and corticosterone appear to have opposite effects on these behaviors. These embryonic injections also alter HPA function. Especially important is the finding

(Martin, 1975) that these corticosterone-treated Pekins responded to immobilization stress at 16 days of age more like wild mallards than like Pekins, *i.e.,* they showed a sustained elevation of plasma adrenocorticoid secretion during stress.

PERSPECTIVES

In this discussion I want to evaluate three questions: 1) does embryonic HPA function influence later approach and/or avoidance behavior in birds; 2) can we attribute the differences in approach/avoidance behavior between wild and domesticated species to differential HPA function during development and 3) how do HPA hormones influence the nervous system that could account for their effects on avian approach/avoidance behavior?

In order to prove that embryonic HPA hormones can act during development to organize the substrate mediating later imprinting and avoidance behavior, three criteria must be satisfied. One must be able to change these behaviors by manipulating embryonic HPA hormone levels, to demonstrate that the embryonic manipulations are physiological rather than pharmacological and to show that the manipulations are specific to the embryonic HPA system. The first of these criteria can be fulfilled. Embryonic injections of small amounts of ACTH (.005 IU) stimulate following (Fig. 3). Larger amounts of ACTH (.5 IU), which would stimulate the embryonic adrenal cortex more effectively for a longer period, do not alter the approach response. Corticosterone-injected ducklings show a different pattern of behavior—in some ways opposite to the ACTH effect. Birds that received the steroid are less likely to approach the imprinting model than controls, and those that do, do so more quickly (Martin, 1975). The slope of the avoidance habituation curve is significantly flatter in the ACTH-treated birds than in vehicle controls; whereas, the corticosterone-treated ducklings show a tendency to a steeper slope than controls. These two hormones produce similar opposite effects in extinction of conditioned avoidance behavior (De-Wied, 1974). The failure of aminoglutethimide to alter approach or avoidance behavior was surprising since the drug did induce a small change in plasma adrenocorticoids 18 days after hatching indicating that it had probably disturbed the embryonic HPA function (Martin, 1973). Metyrapone also failed to influence approach behavior significantly, but did adversely affect retention (Fig. 4).

In the experiments reviewed here it is difficult to establish whether the alterations in the developing embryo are physiological rather than pharmacological, *i.e.,* whether similar changes might obtain due to natural causes. Injections were never made into the embryo *per se,* but into or onto the support tissues. This would permit the hormone to be taken up more slowly at a somewhat constant rate. Over 4 days 50 μg corticosterone might not exceed the secretory capacity of the embryonic duck adrenal since Van der Helm-Hylkema (1973) has shown that the 3-day old rat pup's adrenals can secrete 2.5 μg corticosteroids in 10 hours when stimulated by ACTH. Those ducklings receiving three 50 μg injections weighed significantly less than controls. A lower dose (10+10+20 μg) did not produce growth inhibition, but did affect behavior (Martin, 1975). It seems probable that the lower dose is within a physiological range. Similar considerations apply to ACTH treatment. The lowest dose of .005 IU spread over four days is most certainly physiological.

Present data do not satisfy the third criterion. The actions of the enzyme inhibitors are not specific to the adrenal (Dexter *et al.,* 1967). More importantly, zinc-bound ACTH may remain active *in vivo* for up to 10 days (Laschet and Hohlweg, 1962), and hence the ACTH may have acted directly on the neural substrate during the behavioral test to produce the observed results. Similarly, the duckling may have absorbed any unmetabolized corticosterone present in the yolk after hatching, and this hormone may have influenced the behavior during testing. Martin (1978) has shown that acute injections of corticosterone do reduce ap-

proach behavior; whereas, anti-sera to corticosterone facilitates approach responses. $ACTH_{1-10}$ (Martin and van Wimersma Greidanus, unpublished data), but not ACTH (Martin, 1975), facilitates approach when injected just prior to imprinting. Acute ACTH injections also have no effect on avoidance habituation (Fig. 1). Landsberg and Weiss (1977) report dose dependent effects of acute ACTH injections on imprinting retention in chicks. Doses that produced detectable corticoid secretion blocked retention, but doses which did not activate the adrenals failed to affect retention. However, their data do show that only ACTH-injected ducklings obtained the highest level of retention. Landsberg and Weiss (1977) do not report whether the ACTH injections or stressors influenced approach or following during the training experience. Considering the activational role that HPA hormones have in approach behavior, it is probable that the effects of embryonic injections result from either traces of residual unmetabolized hormone, or from a disturbance in the HPA function induced by the experimental treatment (Jakoubek *et al.*, 1974; Erdösova *et al.*, 1975). Hall and Kalliecharan (1975) found a complex pattern of variation in the embryonic HPA function after injecting cortisone at an early stage, and they caution about drawing quick conclusions from such injection experiments.

It is not clear in what way the HPA hormones exert their activational effects on approach behavior. One possibility is through an action on catecholaminergic pathways. Sparber and Shideman (1968) experimented with chicks which had received reserpine prior to or during incubation. They found inhibition of following behavior when the eggs were treated before incubation, but facilitation of following if the drug was given at 15 days of development. Injection of this catecholamine depleting agent also interfered with later catecholamine levels in the brainstem. ACTH is known to influence catecholamine metabolism in the brain (Versteeg, 1973; Versteeg and Wurtman, 1975), and adrenocorticoids may influence catecholamine degradation (Parvez and Parvez, 1973).

HPA hormones may also act directly to modulate neuronal discharge. Wiggert and Chadar (1975) report that avian brain possesses a high-affinity, limited capacity binding system for glucocorticoids in the optic tectum. In the same general area, Koranyi *et al.* (1969) report increases in amplitude of evoked potentials following cortisol administration and stimulation of the optic chiasma and a diminution in potentials in the same area following injection of $ACTH_{1-10}$.

If, during development, wild ducklings have a more active HPA system than domesticated forms, the injection of ACTH to embryonic Pekins may mimic the mallard phenotype, thus suggesting the contribution of early HPA function to later behavior. ACTH-treated Pekins were like mallards in that they habituated less rapidly in an avoidance test (Fig. 3), although in imprinting behavior they acted less like wild birds. On the other hand, Pekins injected embryonically with corticosterone behaved more like wild mallards in their imprinting behavior. A smaller proportion of these birds than of control birds followed, and they followed more than controls. High levels of both ACTH and corticosterone may, therefore, be necessary to influence the early development and function of the mallard nervous system to produce the characteristic imprinting phenotype. The validation of this hypothesis requires demonstration that the wild genotype produces more ACTH and corticosterone during the embryonic stages, and that reduction of this functional level will produce a Pekin-like phenotype. A demonstration of this latter point would be technically very difficult. Treatment of mallard embryos with metyrapone, which should disturb normal HPA function, produced no significant effects on approach behavior. Although the present data are suggestive, it would be premature to conclude that the differences in early approach and avoidance between wild and domesticated ducklings are strictly attributable to differences in embryonic HPA function.

A major impediment to progress in the kind of studies discussed above is the substantial phenotypic variability encountered in presently available strains of birds. Imprinting studies, in particular, are noted for their high inter- and intrabatch variability (Graves, 1973). Even variations in atmospheric pressure during incubation can affect the behavior (Bateson, 1974). High variability implies many and unknown causal factors; among these causal factors, hormonal modulation may prove to be relatively minor. In one sense, the study of endocrine modulation of avian behavior development is itself premature. The broad endocrine data base present for other laboratory animals is largely absent in birds. Nevertheless, the developing avian system is an attractive one which holds real promise for future research workers.

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