

# Model of Physiological Stress in Chickens 1. Response Parameters<sup>1,2</sup>

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**ABSTRACT** A model was developed to study stress in chickens. Continuous administration of adrenocorticotrophic hormone was employed (ACTH) using physiological mini-osmotic pumps. A validation of controls for this procedure showed that nonhandled (NHCON), sham surgical procedure for pump implantation (SMCON) and surgical implantation of a pump delivering saline (SALCON) were all acceptable controls. Continuous delivery of ACTH at 8 IU/kg BW/d for 7 d caused increases in

plasma corticosterone (CS), glucose (GLU), cholesterol (CHOL), triglycerides (TRI), high-density lipoprotein (HDL), total protein (TP), and the heterophil/lymphocyte (H/L) ratio. Body weight, as well as relative weights of the major immunobiological organs (i.e., spleen, thymus, and bursa of Fabricius) were decreased. Finally, liver was increased due to lipid and moisture accumulation. This model is the first to show in a single experiment all the major adaptive stress responses of chickens.

(*Key words:* stress, model, broilers, adrenocorticotrophic hormone, corticosterone)

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## INTRODUCTION

A variety of stressors have been used to study stress responses in poultry species. These stressors include mediation of the adrenal glands directly by exogenous administration of adrenocorticotropin (ACTH) and exogenous administration of steroid moieties, including corticosterone (CS), cortisone, cortisol, deoxycorticosterone, and dexamethasone. Additionally, various environmental conditions, including hot and cold regimes, have been employed. Other stressors that have been evaluated include injections with various pharmacological preparations, such as reserpine, propranolol, norepinephrine, serotonin, and l-dopa. Social groupings and alterations in population density have been shown to be effective stressors. Finally, feeding various nutritional formulations or toxicants, such as mycotoxins and heavy metals, have been investigated. Several excellent reviews (Frankel, 1970; Freeman, 1971, 1976, 1985; Siegel, 1971, 1980, 1985, 1995) describe the various stressors that have been used to study stress in avian species.

Additionally, Puvadolpirod (1997) recently reviewed stress in poultry species. She evaluated 110 papers con-

cerning the use of 40 different stressors. Juvenile chickens, Japanese quail, turkeys, ducks, and pigeons as well as adult chickens, turkeys, Japanese quail, ducks, and pheasants were employed as experimental subjects. Her review clearly shows that stress in avian species does not constitute a uniform set of adaptive responses that always occurs in a predictable temporal pattern. Conversely, in no single experiment have all the major stress responses ever been shown to occur, much less in a predictable pattern. It is most interesting to note that the majority of these reports concerned juvenile male chickens. Possibly, this phenomenon is because elevated plasma CS and increased circulating heterophil/lymphocyte (H/L) ratio, which are the two most accepted indicators of the stress condition in birds (Siegel, 1995), have never been reported in adult chickens following direct mediation of the adrenals by exogenous administration of ACTH.

In the review by Puvadolpirod (1997), the protocols and results were summarized from 33 papers that employed exogenous ACTH to study stress in avian species. In these reports, dosage of ACTH ranged from a low of 0.25 IU/d for 8 d to a high of 120 IU/kg BW weekly for 3 wk. Routes of administration included i.v., i.p., and i.m. injections, and the carrier vehicle ranged from three different saline preparations to gelatin preparations of several concentrations. There is no accepted route, dosage, or duration of treatment for administration of ACTH to evoke a

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**Abbreviation Key:** ACTH = adrenocorticotrophic hormone; CHOL = cholesterol; CS = corticosterone; GLU = glucose; HDL = high-density lipoprotein; H/L = heterophil/lymphocyte ratio; NHCON = nonhandled control; SALCON = saline control; SMCON = sham control; TP = total protein control; TRBC = total red blood cell; TRI = triglycerides

given set of stress responses in a predictable temporal pattern in poultry species.

Physiological stress responses that occur most frequently in broilers following ACTH treatment include adrenal cholesterol (CHOL) depletion (Siegel and Beane, 1961; Siegel, 1960, 1962a,b; Siegel and Siegel, 1966; Nir et al., 1975; Thaxton et al., 1968, 1982), involution of lymphoid tissues (Garren et al., 1960, 1961; Siegel and Beane, 1961; Thaxton et al., 1982; Gray et al., 1989), lymphopenia (Siegel, 1962a, Thaxton et al., 1968, 1974), elevation of plasma CS (Siegel, 1968; Beuving and Vonder, 1978, 1986; Edens and Siegel, 1975; Beuving et al., 1989), elevation of plasma glucose (GLU) (Siegel and Beane, 1961; Siegel, 1962a,b), elevation of plasma CHOL (Siegel and Siegel, 1966; Siegel, 1968), and immunosuppression of humoral and cell-mediated immune responses (Thaxton et al., 1968; Thaxton and Siegel, 1970, 1972, 1973; Siegel et al., 1983; Murray et al., 1987a,b). It is important to note that this body of work indicates that in no single experiment were all of these parameters affected, nor have all of these been shown to occur in animals within a given experiment.

The literature indicates clearly that a reliable model to study stress in any animal is lacking. The two major criteria of an acceptable stress model are a treatment that is exact and highly repeatable and a predictable set of stress responses that occur in a known temporal pattern. The purpose of this series of papers is to define a stress model in chickens. This model employs continuous delivery of a defined dosage level of ACTH for a defined time period via mini-osmotic pumps. The major stress responses, dosimetry of ACTH, temporal pattern of responses, including digestion and metabolism (responses that have not received sufficient research attention in stressed animals), and a quantitative assessment of stress are included as the components of this model.

## MATERIALS AND METHODS

Peterson × Arbor Acres chicks were used in two separate experiments. Chicks were brooded and reared in floor pens in a curtain-sided broiler house. Pine shavings were utilized as the bedding material in each pen. Continuous lighting was provided by a single incandescent bulb in each pen. Feed and water were provided ad libitum. All birds received a starter diet containing 22.6% protein and 3,135 kcal ME/kg for the first 4 wk; thereafter, a grower diet containing 20% protein and 3,190 kcal ME/kg was fed. These diets are described in Table 1. At 4 wk of age, all female chicks were removed, and only male chicks were included in the experiments. Chicks were identified with plastic leg bands. Besides experimental chicks, each pen contained extra male chicks that received no other treatment. These extra chicks were maintained

TABLE 1. Composition of the diet

Ingredients	Starter diet	Grower diet
Yellow corn	53.93	60.44
Soybean meal	37.29	30.94
Pork fat	4.85	4.77
Dicalcium phosphate	1.84	1.88
Limestone	1.21	1.22
Methionine	0.21	0.88
Salt	0.42	0.42
Vitamin-mineral premix <sup>1</sup>	0.25	0.25
Calculated analysis		
ME, kcal/kg	3,135.00	3,190.00
Crude protein	22.60	20.00
Calcium	0.95	0.95
Total phosphorus	0.64	0.61
Available phosphorus	0.44	0.44
Lysine	1.28	1.10

<sup>1</sup>Supplied per kilogram of diet: vitamin A, 7,700 IU; vitamin D<sub>3</sub>, 2,194.5 IU; vitamin E, 9.9 IU; menadione sodium bisulfite, 2.70 mg; vitamin B<sub>12</sub>, 11 µg; choline chloride, 440 mg; riboflavin, 4.95 mg; niacin, 33 mg; d-pantothenic acid, 8.75 mg; thiamine, 0.99 mg; folic acid, 544.5 µg; biotin, 55 µg; pyridoxine, 0.88 mg; iron, 30 mg; zinc, 55 mg; manganese, 60.5 mg; copper, 7.7 mg; iodine, 1.1 mg; and selenium, 0.22 mg.

to minimize social interactions caused by removal of experimental chicks. All chicks were allowed to acclimate to their experimental environment for 1 wk before experimentation began. Thereafter, experimental chicks were treated according to the design of the experiment.

## Experiment 1

Three hundred chicks were allocated into 20 pens. Each pen contained four experimental birds and 11 extra birds. To validate administration of ACTH by mini-osmotic pumps, four treatments were used. At 5 wk of age, experimental chicks from each of five pens received one of the following treatments: ACTH, nonhandled control (NHCON), surgical sham operation (SMCON), and saline pump implantation (SALCON).

For ACTH treatment, ACTH<sup>5</sup> from a porcine pituitary source was dissolved in 0.85% saline solution. The dosage of ACTH was adjusted to 8 IU/kg BW/d. The hormone was loaded into mini-osmotic pumps.<sup>6</sup> These pumps delivered ACTH continuously at the rate of 1 µL/h for 7 consecutive d. After being filled with ACTH, pumps were implanted subcutaneously on the backs of birds. Feathers and skin of the interscapular tract were sterilized with betadine, and the area was anesthetized locally by injecting 0.1 to 0.2 mL of lidocaine-HCl just under the skin. A small incision (about 10 mm in length) was made in the skin, and a pump was inserted into the opening between skin and muscle. Finally, this incision was closed with one or two surgical staples.

The NHCON birds did not receive pumps and were not sham operated. The SMCON birds were sham operated (i.e., an incision was made and closed without insertion of a pump). The SALCON birds received surgical implantation of pumps filled with 0.85% saline solution.

Measurements used to ascertain the stress response in the first experiment were BW, relative liver weight, and plasma concentrations of CS, GLU, and CHOL. Each pa-

<sup>5</sup>ACTH 1039, Corticotropin A, Sigma Aldrich Fine Chemicals, St. Louis, MO 63103.

<sup>6</sup>Model 2001, Alza Corp., Mountain View, CA 94039-7210.

parameter was measured immediately prior to implantation of the pumps (Day 0) and on Days 4, 8, and 12 postimplantation (Days 4, 8, 12, respectively). At each sampling time, one experimental bird from each pen was measured. In no case was a bird measured more than once.

## Experiment 2

Two hundred forty chicks were allocated into 16 pens, i.e., eight control and eight ACTH-treated pens. Each pen contained six experimental birds and nine extras. The experiment was started when chicks were 6 wk of age. At this time, experimental chicks designated for ACTH treatment received a mini-osmotic pump calibrated to deliver 8 IU ACTH/kg BW/d for 7 consecutive d. The controls did not receive pumps, nor were they sham operated.

On Days 0, 4, and 7 of Experiment 2, two experimental chicks were removed from each pen for sample collections. At no time was a chick sampled more than once. This design provided for eight replications with two subsamples. Thus, 16 ACTH and a like number of controls were sampled at each measurement time.

In Experiment 2, parameters of measurement were BW and relative weights of the liver, bursa of Fabricius, spleen, and third lobe of the thymus on the left side. Liver moisture, lipid, and soluble protein were also measured. Blood cell measurements were total erythrocytes (TRBC) and the ratio of heterophils to lymphocytes (H/L ratio). Blood chemical constituents that were measured included CS, GLU, CHOL, triglycerides (TRI), total protein (TP), and high-density lipoprotein (HDL).

## Blood Samples Prepared

Blood samples, collected by cardiac puncture, were taken within 30 s after each chick was caught. Samples of approximately 10 mL were collected into syringe-needle assemblies that had been flushed with a solution of EDTA. Samples of anticoagulated blood were used for hematological assays in Experiment 2 but not in Experiment 1. The TRBC were determined by the microscopic procedure of Natt and Herrick (1952) and differential leukocyte numbers were determined by the method of Cook (1959). After blood cell determinations, a sample of anticoagulated, whole blood was centrifuged at  $1,800 \times g$  for 15 min. The plasma was collected and stored at  $-20\text{ C}$  for later chemical analysis. Concentrations of all plasma chemical constituents, with the single exception of CS, were determined using an autoanalyzer.<sup>7</sup> This analyzer employs enzymatic procedures that have been described by Elliott (1984). Plasma CS was measured by RIA.

## RIA of Plasma CS

Plasma CS concentrations were determined by RIA with a Coat-a-Count rat corticosterone RIA kit.<sup>8</sup> Binding characteristics of CS are different in rat serum and chick plasma; therefore, standards were prepared by diluting 2,000 ng/mL CS rat serum included in the kit with stripped chick plasma. The stripped chick plasma, with all endogenous steroids removed, was prepared by collecting 200 mL of blood by cardiac puncture. Blood was centrifuged, and plasma was decanted into a 500-mL bottle. Then 25 g of activated charcoal was added and incubated overnight at  $4\text{ C}$  with gentle shaking. Plasma was centrifuged at  $10,000 \times g$  for 10 min and decanted. Another 25 g of charcoal was added and incubated at  $4\text{ C}$  overnight with gentle shaking. Plasma was centrifuged, filtered through Whatman #3 paper, filtered through a  $5\text{-}\mu$  filter, and then filtered through a  $0.45\text{-}\mu$  filter. The stripped plasma was aliquoted and stored at  $-20\text{ C}$ . Assays were performed by adding 200  $\mu\text{L}$  of plasma samples or standards in stripped chick plasma to each tube, followed by 1 mL of the  $^{125}\text{I}$ -labeled CS that was included with the kits. The samples were incubated overnight at  $4\text{ C}$ . The unbound label was aspirated, and the remaining radioactivity was counted on a Cobra™ II Auto Gamma® counter.<sup>9</sup> Inter- and intraassay coefficients of variations were 12.5 and 10.8%, respectively.

## Body and Organ Collection

After blood samples were taken, birds were killed by cervical dislocation. Body weights, as well as weights of liver, bursa of Fabricius, spleen, and the third lobe of the thymus on the left side were recorded. Relative weights of each organ were calculated. In Experiment 2, liver was analyzed for moisture, lipid, and soluble protein content. Liver moisture was determined gravimetrically. Livers were dried for 24 h at  $100\text{ C}$  and then reweighed. Moisture was calculated by difference and is expressed as a percentage of wet liver weight. Total liver lipid was determined by ethyl ether extraction, as described by AOAC (1980). Soluble liver protein content was determined spectrophotometrically.<sup>10</sup> Absorbance at 225 nm was subtracted from that at 215 nm, according to a procedure described by Waddell (1956).

## Statistical Analysis

The experimental design was a completely randomized design in both experiments. In Experiment 1, one-way ANOVA was used to test for treatment effects at each day of sampling. In Experiment 2, repeated measures design was used with treatments arrayed in a completely randomized design in a split-plot in time. All data were subjected to the general linear models procedure of the Statistical Analysis System (SAS, 1990). Means were partitioned by least significant difference (Steel and Torrie, 1980). Statements of significance are based on  $P < 0.05$ .

<sup>7</sup>Ektachem Model DT 60 analyzer, Eastman Kodak Co., Rochester, NY.

<sup>8</sup>Diagnostic Products Corp., Los Angeles, CA 90045-5597.

<sup>9</sup>Packard Instrument Co., Meriden, CT.

<sup>10</sup>Hewlett Packard 8452A Diode Array Spectrophotometer, Hewlett Packard, Avondale, PA.

**TABLE 2. Validation of the efficacy of administration of adrenocorticotrophic hormone (ACTH) (8 IU/kg BW/d) for 7 d via a mini-osmotic pump in Experiment 1**

Parameter or treatment	ACTH (days)			
	Day 0	Day 4	Day 8	Day 12
Plasma CS, <sup>1</sup> ng/mL				
ACTH	0.93 ± 0.58 <sup>a</sup>	20.53 ± 0.14 <sup>a</sup>	10.89 ± 4.75 <sup>a</sup>	1.76 ± 0.69 <sup>a</sup>
NHCON <sup>2</sup>	0.66 ± 0.00 <sup>a</sup>	0.66 ± 0.00 <sup>b</sup>	0.66 ± 0.00 <sup>b</sup>	0.85 ± 0.16 <sup>a</sup>
SMCON	0.66 ± 0.00 <sup>a</sup>	0.66 ± 0.00 <sup>b</sup>	0.70 ± 0.04 <sup>b</sup>	0.69 ± 0.03 <sup>a</sup>
SALCON	0.66 ± 0.00 <sup>a</sup>	0.66 ± 0.00 <sup>b</sup>	0.66 ± 0.00 <sup>b</sup>	1.24 ± 0.58 <sup>a</sup>
Plasma GLU, mg/dL				
ACTH	260 ± 4 <sup>a</sup>	828 ± 140 <sup>a</sup>	789 ± 140 <sup>a</sup>	251 ± 11 <sup>a</sup>
NHCON	250 ± 3 <sup>a</sup>	251 ± 3 <sup>b</sup>	244 ± 3 <sup>b</sup>	242 ± 5 <sup>a</sup>
SMCON	247 ± 6 <sup>a</sup>	260 ± 5 <sup>b</sup>	246 ± 3 <sup>b</sup>	253 ± 8 <sup>a</sup>
SALCON	262 ± 2 <sup>a</sup>	261 ± 4 <sup>b</sup>	246 ± 6 <sup>b</sup>	255 ± 7 <sup>a</sup>
Plasma CHOL, mg/dL				
ACTH	113 ± 2 <sup>a</sup>	200 ± 4 <sup>a</sup>	132 ± 2 <sup>a</sup>	92 ± 7 <sup>a</sup>
NHCON	116 ± 3 <sup>a</sup>	113 ± 5 <sup>b</sup>	92 ± 5 <sup>a</sup>	111 ± 3 <sup>a</sup>
SMCON	102 ± 9 <sup>a</sup>	109 ± 5 <sup>b</sup>	121 ± 3 <sup>a</sup>	115 ± 6 <sup>a</sup>
SALCON	106 ± 6 <sup>a</sup>	134 ± 10 <sup>b</sup>	110 ± 7 <sup>a</sup>	115 ± 7 <sup>a</sup>
BW, g				
ACTH	1,689 ± 69 <sup>a</sup>	1,665 ± 101 <sup>b</sup>	1,814 ± 156 <sup>b</sup>	1,514 ± 167 <sup>b</sup>
NHCON	1,747 ± 69 <sup>a</sup>	1,775 ± 112 <sup>a</sup>	2,200 ± 96 <sup>a</sup>	2,668 ± 78 <sup>a</sup>
SMCON	1,701 ± 68 <sup>a</sup>	1,904 ± 52 <sup>a</sup>	2,277 ± 44 <sup>a</sup>	2,600 ± 128 <sup>a</sup>
SALCON	1,645 ± 71 <sup>a</sup>	2,014 ± 124 <sup>a</sup>	2,337 ± 61 <sup>a</sup>	2,505 ± 217 <sup>a</sup>
Relative liver weight, g/kg BW				
ACTH	34.06 ± 0.60 <sup>a</sup>	48.33 ± 5.88 <sup>a</sup>	52.95 ± 2.67 <sup>a</sup>	50.77 ± 6.01 <sup>a</sup>
NHCON	27.82 ± 1.49 <sup>a</sup>	27.26 ± 0.89 <sup>b</sup>	27.67 ± 1.28 <sup>b</sup>	25.89 ± 1.14 <sup>b</sup>
SMCON	31.34 ± 1.58 <sup>a</sup>	26.31 ± 1.29 <sup>b</sup>	26.69 ± 1.71 <sup>b</sup>	28.08 ± 2.23 <sup>b</sup>
SALCON	30.45 ± 1.37 <sup>a</sup>	29.58 ± 1.05 <sup>b</sup>	28.46 ± 0.93 <sup>b</sup>	25.95 ± 0.60 <sup>b</sup>

<sup>a,b</sup>Means ± SEM within each column with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Sensitivity of the assay was 0.66%.

<sup>2</sup>NHCON = nonhandled control, SMCON = sham control, SALCON = saline control, GLU = glucose, and CHOL = cholesterol.

## RESULTS

### Experiment 1

Results from Experiment 1, which was designed to validate efficacy of delivery of ACTH by mini-osmotic pumps, are shown in Table 2. Overall, among the three control groups (i.e., NHCON, SMCON, and SALCON), no differences were observed in BW, absolute or relative

weights of the liver, or plasma levels of CS, GLU, and CHOL. However, ACTH caused changes in all parameters when compared with the three control groups. Specifically, BW was decreased, absolute and relative liver weights were increased, and plasma levels of CS, GLU, and CHOL concentrations were all increased. All of these changes were consistent through Day 8 of the experimental period. By Day 12, all parameters had returned to the range of values of the three control groups.

**TABLE 3. The effects of continuous delivery of adrenocorticotrophic hormone (ACTH) (8 IU/kg BW/d) for 7 d on BW organ weights in Experiment 1**

Parameter	Treatment	Days postimplant		
		0	4	7
BW (g)	Control	1,616 ± 20 <sup>d</sup>	1,931 ± 35 <sup>b</sup>	2,155 ± 43 <sup>a</sup>
	ACTH	1,718 ± 35 <sup>cd</sup>	1,705 ± 53 <sup>cd</sup>	1,789 ± 63 <sup>c</sup>
Relative liver (g/kg BW)	Control	27.40 ± 1.56 <sup>c</sup>	23.45 ± 0.72 <sup>c</sup>	23.61 ± 0.73 <sup>a</sup>
	ACTH	24.15 ± 0.95 <sup>c</sup>	47.32 ± 2.04 <sup>a</sup>	39.54 ± 3.00 <sup>b</sup>
Relative spleen (g/kg BW)	Control	1.23 ± 0.11 <sup>bc</sup>	1.34 ± 0.09 <sup>ab</sup>	1.54 ± 0.09 <sup>a</sup>
	ACTH	1.48 ± 0.07 <sup>ab</sup>	0.83 ± 0.08 <sup>d</sup>	1.11 ± 0.08 <sup>c</sup>
Relative thymus (g/kg BW)	Control	0.56 ± 0.12 <sup>a</sup>	0.30 ± 0.02 <sup>b</sup>	0.35 ± 0.04 <sup>b</sup>
	ACTH	0.44 ± 0.04 <sup>ab</sup>	0.13 ± 0.01 <sup>c</sup>	0.12 ± 0.02 <sup>c</sup>
Relative bursa (g/kg BW)	Control	0.96 ± 0.08 <sup>a</sup>	0.82 ± 0.06 <sup>ab</sup>	0.89 ± 0.07 <sup>ab</sup>
	ACTH	0.78 ± 0.07 <sup>ab</sup>	0.50 ± 0.04 <sup>c</sup>	0.51 ± 0.04 <sup>c</sup>

<sup>a-d</sup>Means ± SEM within each parameter with no common superscript differ significantly ( $P < 0.05$ ).

**TABLE 4. The effect of continuous delivery of adrenocorticotrophic hormone (ACTH) (8 IU/kg BW/d) for 7 d on liver chemical composition in broiler chicks in Experiment 2**

Parameter	Treatment	Days postimplant		
		0	4	7
Moisture (%)	Control	73.67 ± 0.74 <sup>a</sup>	73.35 ± 0.40 <sup>a</sup>	74.73 ± 0.24 <sup>a</sup>
	ACTH	73.95 ± 0.51 <sup>a</sup>	66.29 ± 0.77 <sup>c</sup>	68.27 ± 1.07 <sup>b</sup>
Lipid (%)	Control	2.68 ± 0.16 <sup>b</sup>	2.77 ± 0.19 <sup>b</sup>	2.31 ± 0.13 <sup>b</sup>
	ACTH	2.41 ± 0.15 <sup>b</sup>	7.56 ± 0.75 <sup>a</sup>	8.73 ± 1.25 <sup>a</sup>
Soluble protein (%)	Control	15.65 ± 0.79 <sup>a</sup>	11.74 ± 0.74 <sup>b</sup>	13.80 ± 0.37 <sup>ab</sup>
	ACTH	14.85 ± 0.77 <sup>a</sup>	11.42 ± 1.77 <sup>b</sup>	12.59 ± 0.44 <sup>ab</sup>

<sup>a-c</sup>Means ± SEM within each parameter with no common superscript differ significantly ( $P < 0.05$ ).

## Experiment 2

The BW and relative weights of the liver, spleen, thymus, and bursa of Fabricius are shown in Table 3. Control chicks grew at a normal rate over the 7-d experimental period and exhibited increases on both Days 4 and 7, as compared with Day 0. However, ACTH-treated chicks did not exhibit normal growth, as evidenced by similar BW at all three times of measurement. As normal growth did not occur in ACTH-treated birds, their BW at Days 4 and 7 were lower than those of the controls.

Relative liver weights (Table 3) in controls did not differ over the experimental period; however, those of ACTH-treated birds were increased at both Days 4 and 7. Relative liver weights in ACTH-treated chicks showed increases of 196 and 165% on Days 4 and 7, respectively. Relative weights of immunobiological organs (i.e., spleen, thymus, and bursa of Fabricius) were decreased on Days 4 and 7 by ACTH.

Liver moisture, lipid, and soluble protein concentrations (Table 4) did not differ in control chicks over the course of the experiment. A single exception was that soluble protein content on Day 4 was lower than that on Day 0. ACTH treatment, however, caused increases in lipid concentrations and decreases in moisture concentrations. Specifically, on Days 4 and 7, concentrations of lipid were elevated, and moisture concentrations were reduced, as compared with values on Day 0. As in the controls, soluble protein concentration on Day 4 was lower than on Day 0.

Results concerning blood cell numbers are shown in Table 5. The TRBC were not elevated at any time by

ACTH treatment; however, there was a decrease in TRBC over time in both control and ACTH-treated birds. Total white blood cells and the H/L ratio were increased at Days 4 and 7 in ACTH-treated chicks, as compared with the controls.

Blood chemical parameters are summarized in Table 6. Values in controls did not differ over the course of the experimental period, except for plasma TP levels. On Day 7, plasma TP was higher than that on Day 0 but not that on Day 4. Also, plasma TP levels on Day 4 were not different from those on Day 0. ACTH, however, caused several effects. Plasma levels of all parameters were elevated on Days 4 and 7, as compared with levels on Day 0, except for TRI levels. This parameter did exhibit elevation on Days 4 and 7, but levels on Day 7 did not differ from levels on Day 0.

## DISCUSSION

Results from Experiment 1 show that no stress-related changes were caused by any of the control treatments. Therefore, the NHCON, SMCON, and SALCON procedures are acceptable controls for the ACTH treatment employed in the present study. A previous report (Davison et al., 1985) supports this conclusion. They found that neither infusion of saline from implanted mini-osmotic pumps nor sham surgical implantation procedure caused stress-related changes.

The results of Experiment 2 indicate clearly that 8 IU ACTH/kg BW/d delivered by surgically implanted mini-osmotic pumps in broiler chicks for 7 consecutive d caused symptoms of physiological stress. Growth, as indi-

**TABLE 5. The effects of continuous delivery of ACTH<sup>1</sup> (8 IU/kg BW/d) for 7 d on blood cells of broiler chicks in Experiment 2**

Parameter	Treatment	Days postimplant		
		0	4	7
Red blood cells ( $\times 10^6$ cell/mm <sup>3</sup> )	Control	2.01 ± 0.08 <sup>a</sup>	1.69 ± 0.04 <sup>c</sup>	1.73 ± 0.03 <sup>c</sup>
	ACTH	1.92 ± 0.05 <sup>ab</sup>	1.83 ± 0.07 <sup>bc</sup>	1.70 ± 0.07 <sup>c</sup>
White blood cells ( $\times 10^3$ cell/mm <sup>3</sup> )	Control	11.31 ± 0.09 <sup>c</sup>	10.72 ± 0.07 <sup>c</sup>	8.75 ± 0.05 <sup>c</sup>
	ACTH	13.75 ± 0.09 <sup>c</sup>	29.72 ± 0.39 <sup>a</sup>	22.72 ± 0.31 <sup>b</sup>
H/L ratio	Control	0.49 ± 0.06 <sup>c</sup>	0.61 ± 0.03 <sup>c</sup>	0.63 ± 0.10 <sup>c</sup>
	ACTH	0.64 ± 0.02 <sup>c</sup>	5.23 ± 1.03 <sup>a</sup>	2.52 ± 0.93 <sup>b</sup>

<sup>a-c</sup>Means ± SEM within each parameter with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>ACTH = adrenocorticotrophic hormone; H/L = heterophil/lymphocyte.

TABLE 6. The effects of continuous delivery of ACTH<sup>1</sup> (8 IU/kg BW/d) for 7 d on plasma chemical constituents of broiler chicks in Experiment 2

Parameter	Treatment	Days postimplant		
		0	4	7
CS (ng/mL)	Control	0.88 ± 0.00 <sup>c</sup>	0.96 ± 0.04 <sup>c</sup>	1.32 ± 0.17 <sup>c</sup>
	ACTH	1.00 ± 0.10 <sup>c</sup>	46.45 ± 5.44 <sup>a</sup>	14.87 ± 5.62 <sup>b</sup>
GLU (mg/dL)	Control	240 ± 3 <sup>c</sup>	235 ± 3 <sup>c</sup>	238 ± 2 <sup>c</sup>
	ACTH	247 ± 4 <sup>c</sup>	878 ± 28 <sup>a</sup>	379 ± 65 <sup>b</sup>
CHOL (mg/dL)	Control	87.38 ± 3.85 <sup>c</sup>	88.13 ± 2.56 <sup>c</sup>	98.50 ± 1.93 <sup>c</sup>
	ACTH	96.50 ± 7.08 <sup>c</sup>	206.63 ± 0.09 <sup>a</sup>	122.13 ± 8.46 <sup>b</sup>
TRI (mg/dL)	Control	94.88 ± 14.44 <sup>b</sup>	92.44 ± 7.47 <sup>b</sup>	83.56 ± 12.01 <sup>b</sup>
	ACTH	81.25 ± 11.53 <sup>bc</sup>	164.06 ± 14.91 <sup>a</sup>	121.44 ± 14.81 <sup>b</sup>
HDL (mg/dL)	Control	63.50 ± 3.09 <sup>c</sup>	69.81 ± 2.14 <sup>c</sup>	75.56 ± 2.27 <sup>c</sup>
	ACTH	67.38 ± 5.82 <sup>c</sup>	140.00 ± 6.54 <sup>a</sup>	95.38 ± 8.08 <sup>b</sup>
TP (g/dL)	Control	2.90 ± 0.09 <sup>c</sup>	3.14 ± 0.07 <sup>bc</sup>	3.41 ± 0.07 <sup>b</sup>
	ACTH	2.96 ± 0.09 <sup>c</sup>	4.09 ± 0.19 <sup>a</sup>	3.96 ± 0.19 <sup>a</sup>

<sup>a-c</sup>Means ± SEM within each parameter with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>ACTH = adrenocorticotrophic hormone, CS = corticosterone, GLU = glucose, CHOL = cholesterol, TRI = triglycerides, HDL = high-density lipoproteins, and TP = total protein.

cated by BW changes, was terminated during the stress period. Liver weight increased, and this increase was apparently due to concomitant accumulations of lipid. Because liver, rather than adipose tissue, is the major site of fatty acid synthesis in chickens (Leveille, 1969), an accumulation of hepatic lipid content is not unexpected because of the metabolic effects of elevated levels of endogenous CS.

The ACTH caused no changes in TRBC numbers. This result is in agreement with the previous work of Siegel (1968) that neither cortisol nor ACTH influenced hematocrits of young chicks. A shift to the right in H/L ratio was pronounced in birds treated with ACTH. These results are in agreement with the previous report of Siegel (1968). As indicated by Gross and Siegel (1983), the H/L ratio was elevated by ACTH throughout the experimental period.

Decreases in relative weights of lymphoid tissues after ACTH treatment are in agreement with previous reports (Siegel, 1962a; Glick, 1967; Davison et al., 1985). Although evaluations of humoral and cell-mediated immune functions were not conducted, based upon the hematological and lymphoid tissue changes, it is predictable that chicks of the present study experienced extensive immunosuppression (Thaxton et al., 1968, 1982).

Evaluations of plasma levels of CS, GLU, CHOL, and TP are in agreement with metabolic changes associated with stress in chickens (Siegel, 1995). The findings of elevations in TRI and HDL at Days 4 and 7, coupled with previous findings of Gould and Siegel (1985) that HDL and very low-density lipoprotein were not elevated at 12 to 18 h post-ACTH treatment, indicate that additional work is needed to understand lipid metabolism during physiological stress in chickens.

In summary, continuous delivery of ACTH caused marked changes in all parameters measured. This study is the first one in chickens in which all of the expected stress changes occurred simultaneously. Although additional work on the dosimetry of ACTH used is required, the results from this study demonstrate that continuous

delivery of ACTH by mini-osmotic pumps is an excellent model to study stress in chickens.

## REFERENCES

- Association of Official Analytical Chemists, 1980. Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists, Washington, DC.
- Beuving, G., R. B. Jones, and H. J. Blokhuis, 1989. Adrenocortical and heterophil/lymphocyte responses to challenge in hens showing short or long tonic immobility reactions. *Br. Poult. Sci.* 30:175-184.
- Beuving, G., and G.M.A. Vonder, 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. *Gen. Comp. Endocrinol.* 35:153-159.
- Beuving, G., and G.M.A. Vonder, 1986. Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and the plasma baseline levels of corticosterone. *Gen. Comp. Endocrinol.* 62:353-358.
- Cook, F. W., 1959. Staining fixed preparation of chicken blood cells with combination May-Grüwald-Wright-Phloxine B stain. *Avian Dis.* 3:272-290.
- Craig, J. V., and J. A. Craig, 1985. Corticosteroid levels in White Leghorn hens as affected by handling, laying-house environment, and genetic stock. *Poultry Sci.* 64:809-816.
- Davison, T. C., B. M. Freeman, and J. Rea, 1985. Effects of continuous treatment with synthetic ACTH<sup>1-24</sup> or corticosterone on immature *Gallus domesticus*. *Gen. Comp. Endocrinol.* 59:416-423.
- Edens, F. W., and H. S. Siegel, 1975. Adrenal responses in high and low ACTH response lines of chicken during acute heat stress. *Gen. Comp. Endocrinol.* 25:64-73.
- Elliott, R. J., 1984. Ektachem DT-60 Analyzer. *Physician's Leading Comput. J.* 2:6.
- Frankel, A. I., 1970. Neurohumoral control of the avian adrenal: A review. *Poultry Sci.* 49:869-921.
- Freeman, B. M., 1971. Stress and the domestic fowl: A physiological appraisal. *World Poult. Sci. J.* 27:263-275.
- Freeman, B. M., 1976. Stress and the domestic fowl: A physiological re-appraisal. *World Poult. Sci. J.* 32:249-256.
- Freeman, B. M., 1985. Stress and the domestic fowl: Physiological fact or fantasy? *World Poult. Sci. J.* 41:45-51.
- Garren, H. W., C. H. Hill, and M. W. Carter, 1960. The effect of fowl typhoid on the response of young chickens to adrenocorticotrophic hormone. *Poultry Sci.* 39:1226-1228.

- Garren, H. W., C. H. Hill, and M. W. Carter, 1961. Adrenal response of young chickens to adrenocorticotrophic hormone as influenced by dosage and frequency of injection. *Poultry Sci.* 40:446-453.
- Glick, B., 1967. Antibody and gland studies in cortisone and ACTH-injected birds. *J. Immunol.* 98:1076-1084.
- Gould, N. R., and H. S. Siegel, 1985. Serum lipoproteins in chickens after administration of adrenocorticotropin or exposure to high temperature. *Poultry Sci.* 64:567-574.
- Gray, H. G., T. J. Paradis, and P. W. Chang, 1989. Physiological effects of adrenocorticotrophic hormone and hydrocortisone in laying hens. *Poultry Sci.* 68:1710-1713.
- Gross, W. B., and H. S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* 27:972-979.
- Leveille, G. A., 1969. In vitro hepatic lipogenesis in the hen and chick. *Comp. Biochem. Physiol.* 28:431-435.
- Murray, D. L., J. Brake, and J. P. Thaxton, 1987a. Effect of adrenocorticotropin and dietary ascorbic acid on cutaneous basophil hypersensitivity to phytohemagglutinin in chickens. *Poultry Sci.* 66:1846-1852.
- Murray, D. L., J. Brake, J. P. Thaxton, and R. P. Gildersleeve, 1987b. Effects of adrenocorticotropin and dietary ascorbic acid on delayed type hypersensitivity to human gamma globulin in chickens. *Poultry Sci.* 66:1859-1969.
- Natt, M. R., and C. A. Herrick, 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poultry Sci.* 31:735-738.
- Nir, I., D. Yam, and M. Perek, 1975. Effects of stress on the corticosterone content of the blood plasma and adrenal gland of intact and bursectomized *Gallus domesticus*. *Poultry Sci.* 54:2101-2110.
- Puvadolpirod, S., 1997. Physiological Stress Responses in Broiler Chicks. Ph.D. Diss., Mississippi State, MS.
- SAS Institute, 1990. SAS/STAT® User's Guide: Statistics. Release 6.04 Edition. SAS Institute, Inc., Cary, NC.
- Siegel, H. S., 1960. Effects of population density on the pituitary-adrenal of cortical axis of cockerels. *Poultry Sci.* 39:500-501.
- Siegel, H. S., 1962a. Age and sex modification of responses to adrenocorticotropin in young chickens. 1. Changes in adrenal and lymphatic gland weights. *Poultry Sci.* 40:1263-1274.
- Siegel, H. S., 1962b. Age and sex modification of responses to adrenocorticotropin in young chickens. 2. Changes in adrenal cholesterol and blood constituent levels. *Poultry Sci.* 41:321-334.
- Siegel, H. S., 1968. Blood cells and chemistry of young chickens during daily ACTH and cortisol administration. *Poultry Sci.* 47:1811-1817.
- Siegel, H. S., 1971. Adrenals, stress and environment. *World Poult. Sci. J.* 27:237-249.
- Siegel, H. S., 1980. Physiological stress in birds. *Bioscience* 30:529-533.
- Siegel, H. S., 1985. Immunological responses as indicators of stress. *World Poult. Sci. J.* 41:36-44.
- Siegel, H. S., 1995. Stress, strains and resistance. *Br. Poult. Sci.* 36:3-22.
- Siegel, H. S., and W. L. Beane, 1961. Time responses to single intramuscular doses of ACTH in chickens. *Poultry Sci.* 40:216-219.
- Siegel, H. S., J. W. Latimer, and N. R. Gould, 1983. Concentration of *Salmonella pullorum* and the immunosuppressive effect of ACTH on growing chickens. *Poultry Sci.* 62:897-903.
- Siegel, H. S., and P. B. Siegel, 1966. Genetic variation in responses to repeated administrations of ACTH and hydrocortisone in immature chickens. *Poultry Sci.* 45:901-912.
- Steel, R.G.D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.
- Thaxton, J. P., J. Gilbert, P. Y. Hester, and J. Brake, 1982. Mercury toxicity as compared to adrenocorticotropin-induced physiological stress in the chicken. *Arch. Environm. Contam. Toxicol.* 11:509-514.
- Thaxton, P., C. R. Sadler, and B. Glick, 1968. Immune response of chickens following heat exposure or injections with ACTH. *Poultry Sci.* 47:264-266.
- Thaxton, J. P., and H. S. Siegel, 1970. Immunodepression in young chickens by high environmental temperature. *Poultry Sci.* 49:202-205.
- Thaxton, J. P., and H. S. Siegel, 1972. Depression of secondary immunity by high environmental temperature. *Poultry Sci.* 51:1519-1526.
- Thaxton, J. P., and H. S. Siegel, 1973. Modification of high temperature and ACTH-induced immunodepression by metyrapone. *Poultry Sci.* 52:618-624.
- Thaxton, J. P., H. T. Tung, and P. B. Hamilton, 1974. Immunosuppression in chickens by aflatoxin. *Poultry Sci.* 53:721-725.
- Waddell, W. J., 1956. A simple ultraviolet spectrophotometric method for the determination of protein. *J. Lab. Clin. Med.* 48:311-314.