

# Effect of Adrenal Blocking Chemicals on Viral and Respiratory Infections of Chickens

Walter B. Gross

## ABSTRACT

In a series of experiments chickens were treated with chemicals which block the production of corticosterone by the adrenal cortex prior to being challenged with respiratory disease (and other) agents in order to determine if the course of the diseases could be altered. Some chickens received a single intramuscular injection (14 mg/kg) of 1,1-Dichloro-2,2-bis/p-chlorophenyl/ethane (ABC) dissolved in corn oil (20 mg/mL) at least 12 h before challenge. Other chickens received feed containing 500 mg/kg of metyrapone for at least 12 h before and during the challenge infection.

Treated chickens were more resistant than the untreated controls to Newcastle disease virus, *Mycoplasma gallisepticum*, combined *M. gallisepticum*-Newcastle disease virus infection, and avian adenovirus group II infection. The feeding of erythromycin (1 g/kg of feed), one day before and during the challenge, reduced the severity of *M. gallisepticum* infection. The effects of feeding both metyrapone and erythromycin resulted in a further increase in resistance. Chickens which had been treated with ABC had less severe lesions and greater postchallenge weight gain than the controls in response to a secondary *Escherichia coli* infection.

## RÉSUMÉ

Cette série d'expériences consistait à administrer à des poulets, des produits chimiques qui inhibent la production de corticostérone par le cortex surrénalien, avant de les soumettre à des

infections de défi avec des agents de maladies respiratoires ou autres, dans le but de vérifier la possibilité d'altérer le cours de ces maladies. Certains poulets reçurent, en une seule infection intramusculaire, 14 mg/kg de 1,1 dichloro-2,2-bis/p-chlorophényl/éthane, dissous dans 20 mg d'huile de maïs/mL, au moins 12 heures avant leur infection. Les autres reçurent des aliments qui contenaient 500 mg de métyrapone/kg, pendant au moins 12 heures avant, ainsi qu'après leur infection.

Les poulets traités se révélèrent plus résistants que les témoins, face aux infections avec les microorganismes suivants: virus de la maladie de Newcastle, *Mycoplasma gallisepticum*, *M. gallisepticum* et virus de la maladie de Newcastle, adénovirus aviaire du groupe II. L'incorporation de 1 g d'érythromycine/kg d'aliments, à compter d'une journée avant l'infection, réduisit la sévérité de l'infection avec *M. gallisepticum*. L'addition de métyrapone et d'érythromycine aux aliments augmenta la résistance à l'infection par ce mycoplasme. Les poulets traités avec du 1,1 dichloro-2,2-bis/p-chlorophényl/éthane développèrent des lésions moins sévères et affichèrent un meilleur gain de poids que les témoins, à la suite d'une infection secondaire avec *Escherichia coli*.

## INTRODUCTION

Chickens which have been subjected to environmental stressors have increased susceptibility to viral diseases such as Newcastle disease, hemorrhagic enteritis and Marek's disease. Stressed

chickens are also more susceptible to *Mycoplasma gallisepticum* infection than unstressed chickens (1-4).

Adrenal blocking chemicals act by blocking the enzyme 11 $\beta$  hydroxylase which mediates the conversion of deoxycorticosterone to corticosterone in the adrenal cortex (5). These chemicals reduce some of the effects of stress on diseases. For example, exposure to social stress increased the incidence of Marek's disease tumors (4). If chickens were also fed dichlorodiphenyldichloroethane (250 mg/kg) the incidence of Marek's tumors was reduced (2). When chickens were close to death from Marek's disease some were fed metyrapone (500 mg/kg). Treated chickens had fewer deaths and more effective cell-mediated immunity than the untreated controls (6).

This study investigated the effect of adrenal blocking chemicals on respiratory and other infections.

## MATERIALS AND METHODS

### EXPERIMENTAL BIRDS

White Leghorn type chickens were from lines selectively bred for either a low (LA) or high (HA) antibody titer response to sheep erythrocytes (7) or for a high (HC) or low (LC) corticosterone response to social stress (8). Chickens were brooded in flocks of from 25 to 32. At four weeks of age each flock was subdivided into flocks of from seven to ten birds which were placed in Horsfall-Bauer type cages where they remained for a four week experimental period. In an effort to reduce variation between cage flocks, chickens from different brooders were

not mixed. A special effort was made to maintain optimal husbandry conditions. The chickens were subjected to minimal human contact.

Turkeys were from a local stock obtained from the Poultry Science Department. Their husbandry was similar to that of the chickens.

#### ADRENAL BLOCKING CHEMICALS

Reagent grade (INC Pharmaceuticals Inc., Plainview, New Jersey) or technical grade (Rothane, Rhom and Haas, Philadelphia, Pennsylvania) 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane (ABC) was dissolved in corn oil and administered as a single intramuscular dose. Doses were 21 mg/kg of body weight for the technical grade product and from 0 to 33 mg/kg of body weight for the reagent grade. When a single dose was employed it was 16 mg/kg of body weight for the technical grade and 14 mg/kg for the reagent grade. Technical grade ABC was used in experiments 6 and 7 while reagent grade was used in experiments 2 and 5.

In some experiments chickens were fed metyrapone (Ciba Geigy, Summit, New Jersey) at the rate of 500 mg/kg for one day before and throughout the challenge period.

#### AVIAN ADENOVIRUS GROUP II

Aliquots of a 10% suspension of spleens from turkeys which were infected with a virulent field isolate were held at -70°C. Turkeys were challenged by administering 1 mL of a 10<sup>-4</sup> dilution of the stock virus orally. Turkeys were examined for intestinal bleeding six days after challenge.

#### NEWCASTLE DISEASE VIRUS (NDV)

Aliquots of chick embryo passed B1 vaccine virus were stored at -70°C. Chickens were exposed to a 10<sup>-1</sup> dilution of stock virus, via the aerosol route by means of a DeVilbiss #40 nebulizer. Tracheal lesions were observed eight days later. Tracheal lesion scores ranged between 0 and 4 on the basis of thickness of the mucosa and the amount of exudate present. A score of 0 indicated no lesions and a score of 4 indicated severe lesions.

Aliquots of a chick embryo passed velogenic strain of NDV (California 11419) were stored at -70°C. Chickens were exposed to a 10<sup>-4</sup> dilution (0.1

mL) via the intramuscular route. Mortality, and number with ataxia four days later, were recorded.

#### MYCOPLASMA GALLISEPTICUM

Aliquots of a culture in Frey's medium were stored at -70°C. Chickens were inoculated, via the air sac route, with 0.1 mL of a 10<sup>-3</sup> dilution of stock culture in tryptose broth. Chickens were examined for air sac lesions 14 days after challenge. Air sacs were scored on a scale of from 0 to 4 with 0 being no lesions and 4 being the most severe lesions, consisting of markedly thickened air sacs covered with thick caseous exudate. The four groups of air sacs were: right posterior thoracic and lesser abdominal, left posterior thoracic and lesser abdominal, left greater abdominal and right greater abdominal. The maximum possible lesion score was 16.

#### ESCHERICHIA COLI

In experiments 6 and 7 chickens were exposed to a 10 h tryptose broth culture of serotype O1:K1 via the aerosol route by means of a DeVilbiss #40 nebulizer. Incidence of death was recorded and three days after challenge surviving chickens were examined for pericarditis and air sac lesions. In evaluating the challenge findings, death was scored 3, pericarditis 2, airsacculitis 1 and no lesions 0. All chickens which died had pericarditis and all with pericarditis also had airsacculitis.

#### LEUKOCYTE CHANGES

The heterophil/lymphocyte (H/L) ratio was used as a measure of the chickens' response to stress (9). Cell counts were made by the hemocytometer method (10) or from slides prepared with May-Greenwald Giemsa stain.

#### EXPERIMENT 1

One day before and throughout avian adenovirus group II challenge 14 five week old turkeys were fed metyrapone and together with 40 controls the birds were subjected to social stress by randomly mixing the 54 birds among eight cages just before the beginning of metyrapone treatment. There were also 18 unstressed, untreated but challenged controls. All turkeys were examined for intestinal bleeding six days later.

#### EXPERIMENT 2

One day before challenge with velogenic NDV 12 chickens were exposed and 12 were not exposed to social stress by moving them among unfamiliar individuals. In another experiment 18 socially stressed chickens were fed metyrapone while 15 were kept as controls. Both groups were challenged with velogenic NDV. Mortality and number with ataxia four days later were recorded.

#### EXPERIMENT 3

In this experiment the effects of time of metyrapone feeding on *M. gallisepticum* infection of LA line chickens were determined. Chickens were fed metyrapone from one day before to two days after, or between five and nine days after *M. gallisepticum* challenge. There were 37 controls and 32 in each of the treated groups. All chickens were examined for air sac lesions 14 days after challenge.

#### EXPERIMENT 4

In this experiment the effects of metyrapone (500 mg/kg of feed) and erythromycin (1 g/kg of feed) on *M. gallisepticum* challenge infection of LA line chickens were investigated. Both treatments were given from one day before and throughout the challenge period. The experimental treatments were metyrapone, erythromycin, both metyrapone and erythromycin and there was an untreated control group. There were between 14 and 20 chickens per group. All chickens were examined for air sac lesions 14 days after challenge.

#### EXPERIMENT 5

In this experiment the effects of ABC on a mixed *M. gallisepticum*-lentogenic NDV infection of 18 HA line chickens were investigated. The ABC was administered 1 day before *M. gallisepticum* was administered via the air sac route and NDV (B1 vaccine strain) was administered via the aerosol route. On the eighth postchallenge day H/L ratios were determined and the severity of respiratory signs was observed. A score of 0 indicates no signs while a score of 3 indicates severe signs. Body weights were recorded, as well as tracheal and air sac lesions.

**TABLE I. Effect of Social Stress and Metyrapone on the Incidence of Intestinal Bleeding Due to Avian Adenovirus Group II Infection**

Level of Social Stress	Group	Incidence of Blood in Gut
High	Control	32/40 (80%) b
	Metyrapone	3/14 (21%) a
Low	Control	4/18 (22%) a

Means followed by different letters are significantly different ( $p < 0.001$ )

**TABLE II. Effect of Social Stress and Metyrapone on the Incidence of Mortality or Ataxia Following Velogenic-Newcastle Disease Virus Challenge**

Level of Social Stress	Metyrapone	Mortality or Ataxia
Low*	No	0/12 (0%) a
High*	No	7/12 (58%) b
High**	No	13/15 (87%) b
High**	Yes	8/18 (56%) a

Means in a column are significantly different ( $p < 0.05$ ) if followed by different letters.

\* = Experiment 1.

\*\* = Experiment 2.

**TABLE III. Effect of Time of Metyrapone Treatment on Air Sac Lesion Scores of *Mycoplasma gallisepticum* Challenge Infection**

Days Metyrapone Administered	n	Mean Air Sac Lesion Scores $\pm$ SD
None	37	1.14 $\pm$ 0.9 c
-1 to 2	32	0.68 $\pm$ 0.8 b
5 to 9	32	0.39 $\pm$ 0.6 a

Means in a column are significantly different if followed by different letters ( $p < 0.05$ )

#### EXPERIMENT 6

In this experiment the influence of dose of ABC on a mixed *M. gallisepticum*-lentogenic NDV-*E. coli* infection was investigated. Chickens from the HA line were injected with 0, 13, 16, 21, 26, or 33 mg of ABC/kg of body weight. Two days later they were exposed to *M. gallisepticum* via the air sac route and NDV via the aerosol route. Eight days after exposure to *M. gallisepticum* and NDV all chickens were exposed to *E. coli* via the aerosol route and body weights were recorded. Three days later all chickens were weighed and examined for pericarditis. There were five chickens in each treatment group.

#### EXPERIMENT 7

In this experiment the effects of the optimal dose of ABC (determined in experiment 6) on a mixed *M. gallisepticum*-NDV-*E. coli* infection were investigated. Nineteen LA line chickens were injected with 16 mg of ABC/kg of body weight one day before they and 18 controls were exposed to both *M. gallisepticum* and NDV via the air sac route. Ten days later H/L ratios were determined and all chickens were exposed to *E. coli* via the aerosol route. All chickens were examined for pericarditis three days later. In another similar experiment there were 30 controls and 16 chickens which were injected with 21 mg of ABC/kg of body weight.

#### STATISTICAL ANALYSIS

Quantitative data were analyzed by means of analysis of variance. When significance was found comparisons among means were made by the Duncan multiple range test. Nonparametric differences were determined by

Fisher's exact test, when numbers were small or by a 2 x 2 contingency table when numbers were larger.

### RESULTS

#### EXPERIMENT 1

Relatively high levels of social stress resulted in an increase in the incidence of intestinal bleeding as a result of avian adenovirus group II infection from 22% in the unstressed controls to 80% (Table I). In the stressed turkeys which also received metyrapone the incidence of intestinal bleeding was reduced from 80% to 21% ( $p < 0.001$ ).

#### EXPERIMENT 2

Social stress resulted in an increase in the incidence of mortality and ataxia following exposure to a velogenic strain of NDV from 0% in the controls to 58% ( $p = 0.005$ ). The feeding of metyrapone to socially stressed velogenic NDV infected chickens (Table II) resulted in a reduction of the incidence of mortality and ataxia from 85% in the controls to 44% ( $p = 0.029$ ).

#### EXPERIMENT 3

The feeding of metyrapone between one day before and two days after the onset of *M. gallisepticum* infection resulted (Table III) in a decrease in lesion scores from 1.14 in the controls to 0.68. Lesion scores were reduced to 0.39 when metyrapone was fed between five and nine days after the onset of infection ( $p < 0.001$ ).

#### EXPERIMENT 4

The feeding of metyrapone resulted in a reduction (Table IV) of the average lesion score of 1.55 following

*M. gallisepticum* inoculation in the controls to 0.89. Lesion score for the erythromycin treated group was 0.69 and for the metyrapone and erythromycin treated group was 0.36 ( $p < 0.001$ ).

#### EXPERIMENT 5

As compared to the untreated controls (Table V) those treated with ABC before a combined *M. gallisepticum*-NDV infection had reduced H/L ratios (0.34 vs 1.02), reduced respiratory signs (1 vs 3), increased body weight (495 g vs 464 g) and reduced tracheal lesion scores (1.1 vs 2.4). Air sac lesion scores were not significantly reduced (4 vs 5.4).

#### EXPERIMENT 6

Compared to those treated with the 0, 13, 26 and 33 mg/kg dose of ABC (Table VI), those treated with the 16 mg/kg dose before a combined *M. gallisepticum*-NDV-*E. coli* challenge infection had a reduced incidence of death or pericarditis (0 vs 50%) ( $p < 0.05$ ). The group which was injected with the 16 mg/kg dose also

**TABLE IV. Effect of Metyrapone and Erythromycin on *Mycoplasma gallisepticum* Challenge Infection**

Group	n	Mean Air Sac Lesion Scores $\pm$ SD
Control	20	1.55 $\pm$ 0.8 d
Metyrapone		
500 mg/kg feed	18	0.89 $\pm$ 0.6 c
Erythromycin		
1000 mg/kg feed	16	0.69 $\pm$ 0.6 b
Metyrapone + Erythromycin	14	0.31 $\pm$ 0.5 a

Means are significantly different if followed by different letters ( $p < 0.05$ )

**TABLE V. Effect of ABC on Respiratory Infection Due to Newcastle Disease Virus and *Mycoplasma gallisepticum***

Parameter	NDV + <i>M. gallisepticum</i>		
	Alone	+ABC	Probability
H/L ratio	1.02	0.34	0.001
Respiratory signs	3	1	-a
Body weight (g)	464	495	0.05
Tracheal scores	2.4	1.1	0.05
Air sac scores	5.4	4.0	NS

<sup>a</sup>Cage observations; no statistics possible.  
There were eight chickens in each group  
NS = not significant

had a greater percent of their original body weight (106%) at the end of the experiment than the uninjected controls (94.6%) ( $p < 0.01$ ).

#### EXPERIMENT 7

Compared to the untreated controls (Table VII) those treated with ABC before a combined *M. gallisepticum*-NDV-*E. coli* infection had reduced H/L ratios (0.56 vs 1.44) ( $p = 0.01$ ) and a reduced incidence of pericarditis (0% vs 60%) ( $p = 0.0007$ ). When the dose of ABC was 21 mg/kg there was no significant decrease in the incidence of pericarditis as compared to the controls.

### DISCUSSION

Increased environmental stress results in decreased resistance to *M. gallisepticum*, NDV, hemorrhagic enteritis virus and Marek's disease virus

(1,3). Treatment with metyrapone or ABC resulted in increased resistance to Marek's disease tumors (2,6), avian adenovirus group II, velogenic and lentogenic NDV, *M. gallisepticum*, and mixed *M. gallisepticum*-NDV infection. Since the adrenal blocking chemicals act by reducing the production of corticosterone by the adrenal cortex, their effectiveness against these infectious agents must be by reducing the stress effects resulting from these infections, or by reducing the chicken's response to the stressfulness of its environment. Similar effects might be achieved by selectively breeding for a reduced corticosterone response to stressors.

The increased resistance produced by ABC or metyrapone treated chickens may be of therapeutic value and in addition may augment the effects of other drugs. One injection with ABC seemed to be long lasting while

**TABLE VI. Effect of ABC on the Incidence of Pericarditis Following Combined Exposure to *E. coli*, *M. gallisepticum* and NDV Infection in LA Line Chickens**

Group	H/L Ratios Before	
	Pericarditis	<i>E. coli</i> Challenge
Control	11/18 (60%)	1.44
ABC	0/9 (0%)	0.56
Probability	0.00007	0.01

metyrapone appeared to be effective only while being administered.

### REFERENCES

1. **GROSS WB, COLMANO G.** The effect of social isolation on resistance to some infectious diseases. *Poult Sci* 1969; 48: 514-520.
2. **GROSS WB.** Effect of social stress on the occurrence of Marek's disease in chickens. *Am J Vet Res* 1972; 33: 2275-2279.
3. **GROSS WB.** The development of "air sac disease". *Avian Dis* 1961; 5: 431-439.
4. **GROSS WB, DOMERMUTH CH, SIEGEL PB.** Genetic and environmental effects on the response of chickens to avian adenovirus group II infection. *Avian Pathol* 1988; 17: 767-774.
5. **DOMINZUEZ OV, SAMUELS LT.** Mechanism of inhibition of adrenal B hydroxylase by metyrapone. *Endocrinology* 1963; 73: 294-309.
6. **THOMPSON DL, ELGERT KD, GROSS WB, SIEGEL PB.** Cell-mediated immunity of Marek's disease virus infected chickens genetically selected for high and low concentrations of plasma corticosterone. *Am J Vet Res* 1980; 40: 91-96.
7. **SIEGEL PB, GROSS WB.** Production and persistence of antibodies I. directional selection. *Poult Sci* 1980; 59: 1-5.
8. **GROSS WB, SIEGEL PB.** Selective breeding for corticosterone response to social stress. *Poult Sci* 1985; 64: 2230-2233.
9. **GROSS WB, SIEGEL HS.** Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis* 1983; 29: 972-979.
10. **GROSS WB.** Differential and total avian blood cell counts by the hemocytometer method. *Avian/Exotic Pract* 1984; 1: 31-36.

**TABLE VII. Effect of Dose of ABC on *E. coli* Challenge of *M. gallisepticum*-NDV Infected Chickens**

ABC mg/kg	Incidence of Pericarditis	Lesion Scores	Percent of Original Weight
0	2/5	1.6 b	94.6 a
13	3/5	1.8 b	97.2 a
16	0/5	1.0 a	106.0 b
21	1/5	1.2 a,b	102.6 b
26	3/5	1.6 b	94.4 a
33	3/5	1.6 b	96.6 a

Means in a column are significantly different if followed by different letters ( $p < 0.05$ )