THE EXPERIMENTAL PRODUCTION OF THE STRESS PICTURE WITH CORTISONE AND THE EFFECT OF PENICILLIN IN YOUNG CHICKENS†

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It is generally accepted that antibiotics will stimulate growth rate in chickens. Several theories attempting to explain this stimulation have been advanced. These include effects on intestinal flora, atypical bacteria, and the endocrines (Freerksen, 1956). A multitude of general and vague statements have been made in the popular poultry literature indicating the antibiotic as an "anti-stress" agent. Since stress "is a state manifested by a specific syndrome which consists of all the nonspecifically induced changes within a biological system" (Selye, 1956), an "anti-stress" agent is an agent that could prevent the conditions of this definition. An "anti-stress" agent could perform its function directly or indirectly, the former by eliminating the harmful organism or stressor agent and the latter by interfering with the cortical hormones released as a result of the stress response. The primary objective of this experiment was to determine the effect of penicillin in the presence of exogenously administered cortisone acetate.

PROCEDURE

The body weight, tissue and white blood cell counts of 72 New Hampshire females were studied from 9 days of age to 37 days of age under four separate treatments. The four treatments consisted of the following: penicillin only, PO; penicillin plus cortisone, PC; basal only, BO; and basal+cortisone, BC. These treatments were randomly assigned to the top four decks of each of 3 batteries. All treatments were, therefore, replicated three times. There were six birds in each deck. The basal diet which was free of medication was fed to all decks for the first nine days after hatching. At this time an initial total and differential white cell count was made of five birds per deck. The Natt-Herrick (1952) technique was employed for the total count and Wright's stain for the differential count. Beginning at 9 days of age penicillin—50 gm/1,000 lb—was added to the feed of the designated groups. One injection of 5 mg of cortisone acetate—0.1 cm^3—was administered to the PC and BC groups at 23 days of age. All groups were bled 3 hours later. At 30 days of age all groups were again bled. Cortisone acetate—5 mg per injection—was administered daily from the thirty-first to the thirty-sixth day of age to the PC and BC groups. All groups were then bled 24 hours after the last injection. It should be noted that a saline control was not employed even though cortisone is suspended in saline solution. According to the recent data of Glick (1958a), a single injection of 0.5 cm^3 saline or multiple injections of 0.1 cm^3 saline will not produce the decrease and increase in lymphocytes and heterophiles, respectively, which characteristically occur following the injection of cortisone acetate. Immediately after bleeding, all birds were killed and their adrenals, thyroids, and bursa were weighed and prepared for later histological examination. Body weights and white blood cell counts were analyzed by the analysis of variance. The analysis of covariance was employed in analyzing the gland weights. Standard errors were calculated for each mean. The statistical procedures were from Snedecor (1946) and Duncan (1955).

RESULTS

The white cell counts are reported in tables 1, 2, and 3. Each mean is based

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on 15 birds. At the start of the experiment none of the white blood cell counts differed significantly, 9-day-old counts. The addition of penicillin to the ration from the ninth to the twenty-third day did not significantly affect the total white blood cells, lymphocytes or heterophil counts (tables 1, 2, and 3). However, the injection of 5 mg of cortisone three hours before bleeding significantly increased total white blood cells, relative and absolute counts of heterophiles, and decreased significantly the percent lymphocytes. The groups receiving penicillin plus cortisone (PC) and basal plus cortisone (BC) were about equally affected by the cortisone injections. There is some indication from the data that the decline in lymphocytes and increase in heterophiles were not as extreme in the PC groups as in the BC groups from the ninth to the twenty-third day (tables 2 and 3). However, too much emphasis should not be placed on this point since two weeks had lapsed from the initial bleeding and differences between the PC and BC groups were not significant. At 30 days of age or one week after one injection of cortisone all groups were bled. The counts were determined to ascertain if the groups receiving cortisone had recovered from their initial stress response. There were no significant differences between the means for total white blood cells, lymphocytes, or heterophiles. That the total counts were not lowered in the PC groups was interesting. There was a trend at 30 days of age for higher lymphocytes and lower heterophil counts in the PO groups as compared to the BO groups. These differences were not significant. From the thirty-first to the thirty-sixth day, cortisone was administered to the designated groups at the level of 5 mg per day (tables 1, 2, and 3). Twenty-four hours after the last injection, the mean total and relative and absolute heterophil counts for the cortisone groups were 7000 cells/mm^3, 15 percent, and 7000 cells/mm^3, respectively, higher than the controls. Also, the mean percent lymphocyte count was 17 percent lower than the control group. All the differences were highly significant at the one percent level. The presence of penicillin apparently had little influence in altering the course of the cortisone injections. However, it should be pointed out that the decrease and increase in percent lymphocyte and percent and absolute heterophiles, respectively, were less extreme in the penicillin groups.

The effect of penicillin plus cortisone on the adrenal, thyroid, and bursa weights is presented in table 4. The data were analyzed by the analysis of covariance. The mean adrenal weight of PC and BC was significantly less than the PO and BO groups. The mean adrenal weight of the PO group was significantly heavier than the BO group. The depressive effect of cortisone on the adrenal gland is no doubt a result of the supression of ACTH production by the anterior pituitary (Ingle, 1938). The stimulating effect of penicillin on the adrenal is not as easily explained. The mean thyroid weights for the four treated groups did not differ significantly. The heaviest thyroids were in the penicillin groups (viz., PO). Cortisone markedly reduced the mean weight of the bursa of Fabricius. Penicillin was unable to protect the bursa against the regressive processes of cortisone acetate. The mean weight of the PO group was heavier than the BO group. The difference was not significant.

Table 5 summarizes body weight gains for the four treatment groups. The groups did not differ significantly in weight gains from the ninth to the thirtieth day. The injection of cortisone during the thirtieth to thirty-seventh day of age significantly reduced weight gain in both BC and PC groups. The PO and BO groups at this time did not differ significantly. Apparently penicillin was unable to protect the bird against the catabolic action of cortisone at the levels injected.

DISCUSSION

The injection of birds with cortisone acetate markedly reduces the relative lymphocyte and increases the relative and absolute heterophil counts (Bannister 1951; Huble, 1955; and Glick, 1958a). The results of this test in respect to cortisone are then in agreement with the avian literature (tables 1, 2, and 3). The addition of procaine penicillin was unable to alter significantly the cortisone produced decrease in lymphocytes and increase in heterophiles. Our data, then, concur with Meites (1952) who reported that an antibiotic in the diet of male rats was unable to prevent the eosinopenic response to cortisone. Since cortisone probably acts directly on the lymphocyte (Dougherty, 1952), an agent that could counteract the effect of injected cortisone would have to interfere directly with the hormone itself, or act as a protector or stimulator at the cell level. Obviously, since cortisone markedly reduced the lymphocyte percent even in the presence of penicillin, neither of our postulates occurred. In the presence of a stressor agent (e.g., an atypical bacterium), glucocorticoids (e.g., cortisone) would be produced by the adrenal cortex (Selye and Heuser, 1956). An antibiotic in the ration would act on the particular stressor agent and thus prevent the stress response. The results would be a lymphopenia in the birds not fed antibiotics and a relatively higher lymphocyte count in the antibiotic fed birds. This explanation may account for part of the white cell increase reported by Glick (1958b) with penicillin.

TABLE 1

The influence of procaine penicillin and cortisone on total white blood cells at various ages in New Hampshire females[1]

	Treatment groups			
	PC	BC	PO	BO
9-day-old	14.2±1.5	13.4±1.1	13.4± .77	12.8±1.0
23-day-old	27.6±2.8	26.2±1.5	18.8±1.1	21.0± .9
30-day-old	27.4±1.6	20.6±1.6	23.4±2.3	24.0±1.5
37-day-old	32.0±1.4	32.0±1.5	23.5±1.3	26.8±1.3

[1]Cells/mm^3 (x1000) ± S.E.
Means not underscored by the same line differ significantly (P<.01) (Duncan, 1955).

Meites (1951, 1952) reported that aureomycin was partially able to counteract the body and thymus weight depression of cortisone acetate. The results of this paper indicate that in the chicken procaine penicillin was unable to prevent the body weight and bursa weight depression which accompanies the injection of large doses of cortisone (tables 4 and 5). It is interesting that the largest bursae were in the PO group. Although the difference between the PO and BO groups was not significant, the results corroborate a past report (Glick, 1957) where significantly larger bursae resulted in the presence of penicillin. According to a review paper (Dougherty, 1952), the thyroid appears to have a slight stimulator effect on certain lymph structures. This might explain the larger bursae of the PO group since the mean thyroid weight was heaviest in this group. In order to verify the thyroid data, a histometrical analysis was made of six thyroids from each group. The height of one epithelial cell in each of four follicles per thyroid was measured under oil immersion with an ocular micrometer. The means and their standard errors are presented in table 6. The histometrical procedure indicated that the PO group may have the most active thyroid. It also rearranged the remaining three groups into the same order as their respective mean bursa weights. The increase in chick thyroid size in the presence of procaine penicillin verifies the work of Draper and Firth (1957).

TABLE 2

The influence of procaine penicillin and cortisone on lymphocytes at various ages in New Hampshire females

	Treatment groups			
	PO	BO	PC	BC
Relative ± S.E.				
9-day-old	79 ±3.7	79 ±2.7	79 ±2.3	82 ±2.5
23-day-old	72 ±2.7	69 ±8.3	61 ±6.4	54 ±4.8
30-day-old	79 ±2.7	76 ±2.1	74 ±5.3	75 ±3.8
37-day-old	76 ±1.8	76 ±2.4	61 ±4.2	57 ±3.4
Absolute x 1000 ± S.E.				
9-day-old	10.5±1.7	10.2±1.4	11.2±1.8	10.7±1.6
23-day-old	13.5±1.2	13.9±1.4	15.6±2.2	14.1±1.7
30-day-old	18.4±2.4	17.9±1.8	20.1±2.3	15.0±1.5
37-day-old	17.9±1.7	19.0±2.4	18.8±1.8	18.3±1.8

Means not underscored by the same line differ significantly (P<.01) (Duncan, 1955).

TABLE 3

The influence of procaine penicillin and cortisone on heterophiles at various ages in New Hampshire females

	Treatment groups			
	BC	PC	BO	PO
Relative ± S.E.				
9-day-old	12 ±2.8	14 ±2.4	14 ±2.9	12 ±4.0
23-day-old	40 ±4.7	35 ±6.5	25 ±5.2	21 ±2.2
30-day-old	17 ±3.3	18 ±3.5	17 ±1.9	14 ±2.1
37-day-old	32 ±2.7	31 ±4.3	14 ±1.6	15 ±2.2
Absolute (x1000) ± S.E.				
9-day-old	1.6± .64	1.8± .46	1.6± .37	1.5± .54
23-day-old	10.4±1.0	10.6±1.3	5.6± .79	3.8± .69
30-day-old	5.4±1.1	4.9±1.3	4.3± .8	3.2± .61
37-day-old	10.5±1.8	10.9±3.2	3.4±1.3	3.6± .74

Means not underscored by the same line differ significantly (P<.01) (Duncan, 1955).

TABLE 4

A comparison of the effect of penicillin and cortisone on the adrenal, thyroid, and bursa weights of 37-day-old New Hamshire females[1]

	PO	BO	PC	BC
Adrenal—grams	.0607 ± .0029	.0521 ± .0024	.0454 ± .0049	.0430 ± .0023
Thyroid—grams	.0481 ± .0035	.0410 ± .0035	.0431 ± .0046	.0409 ± .0040
Bursa—grams	.6058 ± .0596	.5201 ± .0546	.3257 ± .0409	.3753 ± .0400

[1]Eighteen birds per group.
Means not underscored by the same line differ significantly (Duncan, 1955).

TABLE 5

A comparison of the effect of penicillin and cortisone on gain in body weight of New Hampshire females[1]

Gain in grams between	Treatment groups			
	PO	BO	BC	PC
9–23 days of age	208 ± 20	205 ± 8	184 ± 8	207 ± 9
23–30 days of age	118 ± 8	118 ± 9	115 ± 9	109 ± 8
30–37 days of age	163 ± 14	152 ± 18	78 ± 8	68 ± 17

[1]Eighteen birds per group.
All means not underscored by the same line differ significantly (Duncan, 1955).

TABLE 6

Histometrical determination of the thyroids of 37-day-old New Hampshires

Treatment	Epithelial height in microns
Penicillin (PO)	6.27 ± .48
Basal (BO)	5.50 ± .43
Basal+cortisone (BC)	5.18 ± .25
Penicillin+cortisone (PC)	5.04 ± .28

Inhibition of ACTH production as a result of cortisone administration results in adrenal atrophy (Ingle, 1938). In the cockerel and capon, Dulin (1955) was unable to produce adrenal atrophy with twenty 1,000μg doses of cortisone. However, he was able to suppress the adrenal enlargement accompanying epinephrine with cortisone injections. Dulin postulated that the chick adrenal may be slightly independent of pituitary stimulation. The data in table 4 clearly demonstrate that 6 daily injections of 5 mg of cortisone will significantly reduce adrenal weight.

The total amount of cortisone injected exceeded Dulin's by 10 mg. It was obvious from the histological examination that chromaffin cells were in excess of the interrenal cells in the adrenals of cortisone injected birds. We were unable to measure accurately the amount of each cell type in the four groups. The largest mean adrenal weight was in the PO group. Since the largest mean bursa weight was also found in this group, it might suggest that penicillin is able to protect the bursa from slight endogenous increases in adrenal hormones.

SUMMARY

The addition of procaine penicillin to a basal ration—50 gm per 1,000 lb—did not prevent the characteristic decrease in lymphocytes, increase in heterophiles and bursa regression in the presence of cortisone acetate.

Cortisone significantly decreased the weight of the adrenal and bursa of Fabricius. The addition of procaine penicillin significantly increased the weight of the adrenal and bursa. The heaviest mean thyroid was also found in the penicillin group. Penicillin did not affect gain in body weight. Cortisone significantly reduced body weight gains.

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