SOCIAL INTERACTIONS AND ANTIBODY TITRES IN YOUNG MALE CHICKENS (GALLUS DOMESTICUS)

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Abstract. The influence of social interactions on established antibody titres to a Salmonella pullorum antigen was determined in several Athens Randombred families. In two experiments, a large-bodied, aggressive family and a small-bodied, relatively non-aggressive family were placed in three social environments: (1) Four-bird control groups with no changes in membership once formed; (2) three-bird resident groups to which a new fourth bird was introduced daily; (3) the single bird that had been moved to a new group each day. The normal rate of decline in antibody titres was significantly accelerated in birds that were introduced as new members daily (group 3), but only in members of the small non-aggressive family. In a third experiment, using families relatively close in size and aggressiveness, the effects of social grouping on antibody titres were not significant.

Social interactions in flocks of domestic hens are reduced markedly within several weeks of initial assembly (Guhl & Allee 1944; Siegel & Hurst 1962). By contrast, in flocks with rotating membership there is an increased frequency of social interactions manifested by a greater divergence in pecking activity among individuals that vary in social rank (Guhl 1968). A similar increased frequency of social interactions occurs with fighting cocks where the incidence of fighting increases when birds that had been in the same flock are separated and then brought together again (Schjelderup-Ebbe 1935).

Hypophyseal-adrenal activity increases as social tension increases in mammals (Christian 1959; Vandenbergh 1960) and in fowl (Siegel & Siegel 1961); adrenocorticotropic (ACTH) and adrenal steroids depress antibody titres in chickens (Glick 1967). We wanted, therefore, to determine whether increased social interactions would influence the rate of disappearance of antibodies from the blood sera of young chickens.

Methods

Pretreatment Period

Cockerels were obtained from sire families of sublines of Athens Randombred chickens that had been maintained as a closed flock at the station for seven generations. They were groups of half-brothers sired by four males each mated to at least fifteen females. The sublines originally had been separated on the basis of high (HR) and low (LHR) responsiveness to ACTH. For experiments 1 and 2, the sire families that were chosen on the basis of observation of behaviour in the previous generation were: a large-bodied, aggressive family (8-HR); a small-bodied, less aggressive family (7-LR). For experiment 3, the two families used were of approximately equal size and aggressiveness (Families 2-LR and 4-HR).

The chicks were brooded from 1 day to 3 weeks of age in heated metal batteries and then were housed in isolation cabinets of a type described by Drury, Beard & Hopkins (1969) with inside dimensions of 60 x 60 x 46 cm.

Measurement of Agglutinating Antibody

At 6 weeks all birds were immunized with two intravenous injections of Salmonella pullorum antigen (polyvalent K, density 50 x No. 1 on the MacFarland scale). The first injection was 0.25 ml/bird and the second, given 3 days later, was 0.50 ml/bird. Agglutinin titres were determined on log2 dilutions of sera by using a microtitre technique (Thaxton, Williams & Siegel 1970) and a stained antigen (Williams & Whittemore 1971). Serum agglutinating antibodies against S. pullorum were permitted to reach maximum levels before social treatments were begun.

Social Treatments

At 7 weeks, the cockerels were divided into three treatment groups: (1) Controls: four-bird groups that were handled daily, but into which no new members were introduced; (2) Residents: three-bird groups into which a new fourth member was introduced daily; (3) Visitors: the new member that was introduced. Visitors were moved daily from group-to-group for 11 days according to a predetermined schedule that
minimized the return to a familiar cabinet. All bird assignments were made by tables of random numbers with the restriction that equal numbers of each sire family be represented. Specific numbers of cabinets used in each experiment will be given with the results.

**Behavioural Observations**

The number of pecks and visual threats delivered were recorded by observing each group for 10 min per day through 14-cm² windows fitted in the door of each cabinet. Cabinets were interiorly lighted, but the observer was in darkness so that he was not visible to the birds. Feed was removed 8 hr before and returned to each cabinet just before the 10-min observation period. This tended to increase the degree of aggressive pecking during observation and also tended to concentrate the area under observation to the proximity of the feed trough.

**Sampling and Data**

One-ml blood samples were taken from the brachial vein of each bird for agglutinin titration just before the first antigen administration at 6 weeks, just before the initiation of social treatments at 7 weeks, and then every other day during the treatment period. Titres for each bird at each bleeding time were converted to the percentage of decline from his pretreatment titre, and linear regressions were computed. Differences among regression coefficients (b) were analysed according to Snedecor & Cochran (1968). Body weights and gains during the experimental period were subjected to analysis of variance before differences among means were examined by Kramer's (1956) modification of the multiple-range test.

**Results**

**Experiment 1**

At 3 weeks, twenty-four males each from sire families 7 and 8 were assigned, two per family, to twelve isolation cabinets. When the social treatments began, at 7 weeks of age, four cabinets were designated as controls and the other eight as resident-visitors. In each of the latter cabinets, one bird was assigned as visitor and the other three as residents. Visual observations were made for 10 min per chamber per day in two 4-day periods.

The plot of aggressive behaviour by days of observation (Fig. 1) shows that daily movement of strangers into organized flocks resulted in a higher number of pecks delivered. This was attacking behaviour, not casual pecking, and usually the recipient showed avoidance behaviour by turning its head into a corner or hiding under the feeding trough. Except on day 7 there were significantly greater numbers of pecks (P < 0.01) delivered during each observation period in cabinets where birds were moved than among the controls. In this experiment, we did not record whether the attacking behaviour was committed by visitor or by resident or what family was involved.

The mean log₂ pretreatment antibody titre for this experiment was 7.5 ± 0.5. Figure 2 shows the linear regressions depicting declines in agglutinin titres to *S. pullorum* antigen over the 11-day treatment period for both families. A comparison of the 'b' coefficients indicates that the rate of decline of serum agglutinins was significantly greater for the visitors of family 7 than for the controls of that family or for controls and visitors of family 8 (P ≤ 0.05) and was significantly greater than that of the residents of family 8 (P ≤ 0.01). The 'b' coefficients of the residents of family 7 were intermediate to the controls and visitors of that
Fig. 2. Linear regressions depicting declines in primary antibody titres of *S. pullorum* antigen over the 11-day treatment period. Experiment 1. b values ± sE with different superscripts are significantly different (P < 0.05).

Table I. Effect of Social Interactions on Body Weights of ARB Males: Pretreatment Weight, Post-Treatment Weight, and Weight Gains in (grams): Experiment 1

| Family  | Resident | Visitor | Controls | Family 7 |  | Family 8 |  |
|---------|----------|---------|----------|----------|  |----------|  |
|         | Pretreat. wt. | Posttreat. wt. | Wt. gain | Pretreat wt. | Post-treat wt. | Wt. gain |
| Resident | 965 ± 28 | 1188 ± 32 | 223 ± 10 | 1067 ± 31* | 1301 ± 35* | 233 ± 8 |
| Visitor  | 968 ± 58 | 1136 ± 70 | 167 ± 25 | 1111 ± 52* | 1355 ± 66* | 244 ± 15* |
| Controls | 983 ± 51 | 1213 ± 60 | 230 ± 12 | 1090 ± 32* | 1335 ± 53* | 245 ± 21 |
| Family 8 | 972 | 1179 | 207 | 1089 | 1330 | 241 |

*Treatment Means ± sE between families within weighings are significantly different (P ≤ 0.05). Underlined value is significantly different than the others in its column (P < 0.05).

family. Significant differences were not found among the 'b' coefficients of family 8, which indicates that rates of decline of antibody titres for that family were approximately parallel. Note also that the b's of the controls of the two families were not significantly different.

Body weights and gains for experiment 1 are shown in Table I. Both pretreatment and
post-treatment weights were significantly lower in all treatment groups of family 7 than in those of family 8; however, the gain in weight over the 11-day period was lower only in the visitors of family 7.

**Experiment 2**

In this experiment, birds were individually marked by sire family and treatment during the observation period. Thirty-six males from family 7 and thirty-six from family 8 were housed in eighteen isolation chambers arranged as two replicates. Visual observations were for 10 min per chamber per day in one 4-day and one 5-day period. Age at housing, social treatments, immunizations, samplings and titrations were the same as those of experiment 1.

Numbers of pecks delivered by each of the social treatment groups for each of the 9 days of observation may be seen in Fig. 3. As observed previously, the lowest level of aggressive pecking behaviour was found in the controls. Between the visitors and residents, there were no significant differences until day 4, when the numbers of pecks delivered by visitors were significantly lower. During the second week, aggressive pecking was observed by visitors only on days 9 and 10. Distribution of daily pecking by visitors and families can be found in Fig. 4.

Note that on each observation day except day 3, visiting males of family 8 delivered a significantly greater number of pecks. During the second week, no aggressive pecking by visitors of family 7 was observed during any 10-min period.

Shown in Fig. 5 are plots of the regressions of percentage of pretreatment agglutinin titres by day of treatment. The actual mean log₂ pretreatment titre was 7.3 ± 0.3. The slopes for both visitors and residents of family 7 were significantly greater than those for the visitors of family 8 ($P < 0.01$). Again, as in experiment 1, rates of decline in titres of controls of both families were nearly identical.

**Experiment 3**

This experiment was performed to determine the effects on agglutinating antibodies when families of similar body size were used. The design was essentially the same as that of experiment 2 except that thirty-two birds of family 2 and thirty-two of family 4 were placed in sixteen isolation chambers at 3 weeks of age.

Figure 6 shows the data for aggressive pecking by day of observation. There were fewer pecks per bird per 10-min observation among controls than among residents throughout the 11-day period. After the second day, pecks by visitors were significantly fewer in number than those by residents. The distribution of pecking activity by visitors within families (Fig. 7) indicates that after days 1 and 2, during which visitors of family 4 showed significantly higher levels of
aggressive pecking than did visitors of family 2 \((P \leq 0.05)\), there were no significant differences between the two families. Differences in body weight or differences in weight gains between families or treatments were not significant in this experiment.

Figure 8 shows that rates of decline in titre did not differ significantly. Although the mean log₂ pretreatment titre was lower in this experiment \((5.4 \pm 0.3)\), note that the regression coefficients were as high as or higher than those of previous experiments (see Figs 2 and 5).

**Discussion**

Social tension and the functioning of the hypophyseal-adrenal system are related. Christian (1959) and Vandenberg (1960) have shown that hypophyseal-adrenal activity increases as social tension increases among mammals, and a similar relationship has been observed in fowl by Siegel & Siegel (1961). Gross & Siegel (1965) reported that resistance to pathogenic strains of *Escherichia coli* and *Staphylococcus aureus* increases when the social structures of groups of young male chickens are disrupted by the daily moving of visitors into socially established groups. Gross & Colmano (1969) have reported, however, that resistance to *Mycoplasma gallisepticum* and Newcastle disease virus declines with such treatment. Gross & Colmano (1967, 1970) cited increased plasma corticosterone as evidence that these changes in resistance may be related to adrenal function and that increases or decreases in resistance depend on the type of disease organism involved.

Adrenal steroids, either from exogenous sources or as the result of stress, influence antibody levels in mammals and birds. Smith, Sherman & Middleton (1972) have shown that hydrocortisone depressed immunoglobulin synthesis and secretion in human peripheral lymphocytes. Injections of adrenal steroids or ACTH depress antibody titres in chickens (Glick 1967) and pharmacological interference
with normal adrenal corticosterone synthesis prevents or modifies normal immunodepression that results from heat stress (Thaxton & Siegel 1973).

The first two experiments reported here show that the rate of decline in serum agglutinins was accelerated in groups of chickens where new members were introduced daily but that this occurred in only one of the sire families. Titres declined most rapidly in the sera of birds from the small-bodied family that were moved from group to group and which showed consistently lower aggressive pecking activity. When sire-families were of approximately equal body size and displayed similar aggressive pecking activity (experiment 3), differences in decline rates of agglutinating antibody were not significant.

If declines in antibody titre are related to the activity of the hypophyseal-adrenal complex in chickens, as suggested by the results of Glick (1967) and Thaxton & Siegel (1973), one might expect that the cockerels of family 8 would show the more dramatic declines, because they had been originally separated on the basis of their greater responsiveness to ACTH. The observations of behaviour indicated, however, that members of family 8 also delivered most of the agonistic pecks and therefore were probably in a less stressful state.

The results of these experiments suggest that the decline in titres was related to the level of agonistic behaviour within the group. The movement of individuals into new groups resulted in daily initial encounters that tended to suppress social inertia and prevented the establishment of stable peck-orders (Guhl...
The higher frequency of social interactions would favour the large-bodied individual that is more successful in initial encounters (Guhl 1962). In the control flocks where peck-orders were undisturbed and levels of aggressive pecking activity low, the rates of decline in antibody titres in the two families were low and nearly identical. This result suggests that in chickens the relationship between peck-rank and physiological stress may not be apparent unless levels of social interactions are high. Similarly, Siegel & Siegel (1961) showed that chickens lower in the peck-order had heavier adrenals only when they were placed with flocks for short periods each day. In those grouped continuously, this relationship was not observed.

The differential rate of decline in titres between families, when individuals were introduced as new flock members each day, represented a significant source of variation. Similar genetic × environment interactions also have been demonstrated for reproductive traits in strains of chickens selected for high and low social dominance (Biswas & Craig 1970). The presence of such interactions may obscure important effects in behavioural-physiological research.

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REFERENCES


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