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**Genetic Factors Affecting the Rate of
Development of Immunological Maturity
With Respect to Disease Agents in
Poultry**

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B. TABLE OF CONTENTS

	<u>Page</u>
C. ABSTRACT	1
D. BODY OF THE REPORT	
D.1 Materials and Methods	2
D.2 Results	10
D.3 Discussion and Conclusions	35
E. Evaluation of the research achievements	38
F. Description and results of cooperation	39
G. Theses	39
H. Benefit to Agriculture	39
I. Inventions	39
J. Publications	39

C. ABSTRACT

1. Developmental patterns of early immune response to E. coli and Sheep red blood cells (SRBC) was found to differ between light (Leghorn, Leghorn x New Hampshire) and heavy breeds (White Rock) of chicken. Light breeds showed more rapid early development of immune response to E. coli, but reached a final level below that of the heavy breeds. Light and heavy breeds showed similar early development of immune response to SRBC, but light breeds plateaued before heavy breeds.
2. Macrophage activity was greater in light breed chicks than in heavy breed chicks at the time of greater immune response.
3. Two-way sire-family selection for high and low early immune response to E. coli and NDV vaccine, based on an index of the mean titer to both antigens gave a strong response, in two separate replicates.
4. In a comparison to two commercial strains, the low line was clearly low, the high line suggestively high.
5. Actual antibody titers in high and low lines differed by an amount that could have significant consequences on exposure to the disease agent.
6. Overall mortality, and mortality on specific exposure to E. coli was less in the high line than in the low line.

D. The body of the report

Two series of experiments were carried out. The first was a series of comparisons of immune response to E. coli or sheep red blood cells (SRBC) in layers (Leghorn or Leghorn x New Hampshire cross) as compared to White Rock broiler strains. In some cases the comparisons also included tests at a cellular level of number of plaque forming cells (PFC), rate of phagocytosis and rate of killing by macrophages. The second series of experiments included two selection experiments for high and low response to E. coli and NDV antigens. Selection was on the basis of an index (Titer Index): The mean titer to both antigens. The first selection experiment (Replicate A) is still continuing and has completed three cycles of selection. The first two cycles are reported here. The second selection experiment (Replicate B) was carried out for one cycle of selection only.

D.1 MATERIALS AND METHODS

D.1.1 Strain Comparisons

Chicks: The following breeds and strains of chickens were used.

- (1) White Leghorn layer, (2) Layer strain cross (Leghorn x New Hampshire
- (3) White Rock strain 1 (Israel Poultry Breeders Union ANAK80 line),
- (4) White Rock strain 2 (Yavne Poultry Breeders White Rock), (5) Broiler (White Rock x Cornish Cross).

All chicks were housed in brooder batteries, with unlimited access to food and water for the duration of the experiments. Batteries were heated until 4 weeks of age.

Vaccines

E. coli vaccine; killed E. coli (serotype 078:K80) served as antigen. The bacteria were grown on Nutrient Broth at 37°C for 24 hours. The culture was washed 3x with physiological saline solution (PBS). After the last washing the bacteria were resuspended in PBS to a final concentration of 1×10^8 /ml. Before immunization the suspension was boiled for one hour. The immunization was carried out by I.V. injection.

Sheep red blood cells: A suspension of sheep red blood cells washed three times in PBS and resuspended at a final concentration of 20%. Immunization was by 0.2 ml of the suspension.

Passive hemagglutination test (Sojka, 1965)

The test is based on the fact that erythrocytes can adsorb to their outer surface, antigenic fractions of microorganisms, and under appropriate conditions the erythrocytes will be agglutinated in the presence of antibodies to the adsorbed antigens.

To prepare the antigen E. coli bacteria were grown overnight on NB and 3 ml of the bacterial culture was transferred to Roux bottles with Agar substrate. The culture was incubated for 48 hours at 37°C. After incubation 10 ml of PBS and sterile glass beads were added to each bottle. The bottles were shaken until the entire bacterial culture went into suspension. The bacterial suspension was collected in an Erlenmeyer flask and boiled for one hour. After boiling the suspension was centrifuged for 30 minutes. The supernatant containing the antigen was collected in test tubes and kept at 4°C for 3-5 days.

To prepare sensitized erythrocytes, blood was collected from the jugular vein of healthy sheep and kept at 4°C. From this blood, washed cells were prepared at the day of test. To 1 ml of washed erythrocytes were added 5 ml of antigen solution and 95 ml of PBS. The mixture was

incubated 45 minutes at 37°C. The erythrocytes were then washed 3 times in 20 volumes of PBS. After the last centrifugation the cells were resuspended to a final concentration of 0.75-1%. At this concentration the cells are ready for the passive hemagglutination test.

The serum for testing was obtained by bleeding the birds 6 days after immunization. The serum was incubated at 56°C for 30 minutes. Serial dilutions of the serum were carried out in microtitration plates. The first row included PBS only as a negative control. To each well 50 microliter of sensitized erythrocyte suspension was added. Each test was carried out in duplicate. The plates were kept for one hour at room temperature, and then in the refrigerator overnight. In a positive test the erythrocytes were observed settling as a disc, while in a negative test the erythrocytes settled as a pellet in the center of the well, and returned to suspension on shaking the plate.

Rate of phagocytosis

In order to obtain peritoneal macrophages, birds were injected with Sephadex producing an intraperitoneal inflammation. After 72-96 hours the birds were anesthetized with nembutal, and the peritoneal cavity washed with Hank's Balanced Salt solution (HBSS), to which heparin, 1000 IU/100 ml solution, and an antibiotic were added. The peritoneal washings were centrifuged for 6 minutes. The cells were resuspended and washed 2x with HBSS + antibiotic, and brought to a concentration of 10^6 /ml. The cell suspension was then added in 5 ml aliquots to plastic dishes, and incubated at 37°C in 5% CO₂. After 1 hour most of the macrophages had adhered to the bottom of the plates and the remaining cells were removed by washing once in HBSS + antibiotic. 5 ml of FCS 10% + RPNI + antibiotic was added to each plate and the plates were incubated overnight.

The following day cells were removed from the bottom of the plates

by 0.25% trypsin solution. The cells were washed twice in HBSS and then suspended in FCS 10% + RPMI, and incubated with bacteria for 2 hours.

The bacteria for incubation with peritoneal macrophages were inoculated in broth the day before and incubated overnight. They were washed 3 times in PBS and brought to a final concentration of 10^7 /ml. The bacteria were incubated for 30 minutes at 37°C with serum containing inactivated antibodies (Bacterial:serum ratio, 1:2). The serum was obtained from a bird immunized 6 days previously with E. coli antigen, inactivated, and then filtered through a sterile filter.

Two ml of macrophage suspension, 10^6 concentration, was incubated in a water bath at 38°C with 1 ml bacteria, 10^7 concentration. Samples were taken at 0, 30, 60, 90, and 120 minutes. The bacteria samples were diluted and spread on MacConkey agar for counting.

The rate of phagocytosis, K, was calculated by

$$K = \frac{\log C_2 - \log C_1}{t_2 - t_1}$$

where C_2 and C_1 are the bacterial concentrations at times t_2 , and t_1 , respectively. K is thus the slope of log bacterial concentration against time.

Determination of bacterial killing by macrophages:

Peritoneal macrophages were obtained as above. After overnight incubation at 37°C they were incubated 20 minutes with E. coli cells to allow phagocytosis. Excess bacteria (not phagocytosed) were washed away in the supernatant. The supernatant samples were spread on MacConkey plates. When the supernatant contained cells and phagocytosed bacteria only, it was incubated for a number of hours. Each 45 minutes a sample was taken. The cells were burst by 0.5% Deoxycholate and spread on MacConkey plates in order to count surviving bacteria. Rate of killing was determined by the K-index as above.

Plaque forming cells (PFC)

The chicks were injected with 0.2 ml of 5% SRBC solution. After four days the spleen was removed, minced and white blood cells collected. SRBC (0.1 ml, 20% solution) and whiteblood cells (0.1 ml, 3×10^6 cells) were mixed with Indubiose agar and poured on a petri dish. The dish was incubated at 37°C for 40 minutes. Chicken blood serum was added to provide complement, and the dish was returned to the incubator for 30-60 minutes, until first appearance of plaques. The remaining serum was then removed, and the dish refrigerated overnight. Plaques were counted the following morning.

Immune response to NDV

Viruses. Attenuated and inactivated vaccines were prepared from the same stock of virus: the Komarov attenuated (mesogenic) strain (4), grown in the allantois of chicken embryos. The virus titer was $10^{9.3}$ ELD₅₀/ml. The virus was inactivated by adding beta-propiolactase to the virus at a final concentration of 1:1000 and allowing the mixture to stay at 37°C for 3 hours. Virus viability after inactivation was tested in chicken embryos. Al(OH)₃ gel was then added to constitute 50% of the vaccine and served as adjuvant. Both kinds of vaccine were administered intramuscularly. The dose of attenuated vaccine was 0.2 ml/chick, containing $10^{6.5}$ ELD₅₀. The dose of inactivated vaccine was 1 ml/chick, containing $10^{9.0}$ inactivated ELD₅₀. The same virus strain was used for hemagglutination (HA). Challenge of the chicks was by a velogenic strain isolated in Israel in 1967 and grown in chick embryo allantois.

Sera and erythrocytes. A pool of hyperimmune sera was obtained from chicks vaccinated with the Komarov strain and challenged 2 weeks later with the velogenic strain. These chickens were bled 2 weeks after challenge, and their sera pooled and stored at 4°C pending titration. For the hemagglutination-inhibition tests (see later) rooster erythrocytes were pooled and washed three times with phosphate-buffered saline, pH 7.2, and the packed cells were stored at 4°C.

Challenge. On day 12 after vaccination, the experimental chicks were challenged with 0.5 ml of virus containing $10^{5.3}$ ELD₅₀. Mortality and morbidity among the birds was observed during 14 days. Mortality was considered specific from the third day after challenge. Included in the mortality count at the end of the observation period were morbid birds showing nervous symptoms such as torticollis.

Hemagglutination-inhibition test (HI). Antibody response was evaluated by titrating the HI antibodies by the beta-microtest procedure. The test was carried out in U-bottomed plates. Twofold dilutions of serum in PBS were made in 0.05 ml. The concentration of erythrocytes was determined by using a Coleman Junior Spectrophotometer, model 6A, at a wavelength of 4900 nm. The working suspension was brought to 0.5 optical density (OD). A fixed amount of antigen (4 hemagglutinin units in 0.05 ml) was added to the serum dilutions, the mixtures were slightly shaken and allowed to stay at room temperature for 20 minutes, and then 0.05 ml of erythrocytes was added. The results were read after 90 minutes at room temperature. All sera were routinely titered twice and occasionally three times. In most cases results were identical. The first titer only, was therefore used in the statistical analyses.

D.1.2 Selection lines

The selection experiment was carried out in two replicates. The first replicate (Replicate A) is an ongoing selection experiment that has now reached the S-4 generation. The second replicate (Replicate B) was a one step experiment carried out in order to confirm the strong response to selection observed in Replicate A. In both replicates selection was based on sire family averages. Chicks from each family were tested separately

for immune response to E. coli and NDV and sire families were chosen on the basis of mean family titer to both antigens (Titer Index).

Replicate A

The foundation flock was hatched on November, 1979 out of a White Rock population of the Israel Poultry Breeders Union ANAK-80 strain. Twenty-five sire families were established, and four hatches were obtained in September-October, 1980, 550 chicks in all. Hatches 1 and 3 were tested for immune response to E. coli at the Faculty of Agriculture. Hatches 2 and 4 were tested for immune response to NDV at the Veterinary Institute. Data were summarized and family Index Values calculated. On the basis of the Titer Index values 8 families with highest Index Values were chosen to initiate the high line and 8 families with the lowest index values were chosen to initiate the low line. Males and females were retained from each of the chosen families to produce the S-1 generation.

The S-1 chicks were hatched in October-November, 1981 in a total of 7 hatches, 760 chicks in all over both lines. Chicks were tested for immune response to E. coli and to NDV as above. Final results of the tests of the S-1 chicks were obtained by December 1981, and selections were carried out among the S-1 generation, within the high line and low line, to choose the parents of the S-2 chicks. Four males and 24 females were retained in the high line and the same number in the low line. Additional males and females were introduced into each line, in order to increase the number of families for testing.

Inseminations to produce the S-2 chicks were begun in March 1982. Eleven hatches were obtained between May-October 1982. Offspring were obtained from 6 males and 64 females in the high line and from 8 males

and 64 females in the low line. 860 chicks in all. The chicks were tested for immune response to E. coli and NDV as above. When the data were summarized family selection was carried out, and 10 males and 30 females were retained in each line.

In March 1983 the S-2 selected birds were inseminated, and hatches of S-3 chicks were obtained between May-July 1983, seven hatches and 500 chicks in all. On the basis of the S-3 results three families were chosen in the high line and three families in the low line. One male and 10 females were retained from each family. At the time of writing this report the S-4 chicks have just been hatched and tested for immune response.

Replicate B

In May 1982 a second series of families was established from direct descendants of the original flock. Thirty sire families were established, and 900 chicks obtained in two hatches. The chicks were tested for immune response to E. coli and NDV, as above. On the basis of family Index Values six high line families and six low line families were defined, and 12 males and 60 females retained in the high line, the same number in the low line. Inseminations were carried out in February 1983, and chicks were obtained in six hatches between March-June 1983, a total of 760 chicks. These chicks were tested for immune response to E. coli and NDV in order to determine the response to one generation of selection. The lines were discontinued after the results of the S-1 chicks were obtained.

D.2 RESULTS

D.2.1 Strain comparisons

A series of four experiments was carried out. These will now be described and their results summarized.

Experiment 1

The purpose of this experiment was to compare immune response in male and female chicks. The experiment was carried out on White Rock strain 2 chicks. Chicks were immunized with E. coli vaccine in groups of 13-18 birds, on days 0, 14, 28, and 42 after hatching. Each chick received 10^8 bacteria. Table 1 shows antibody titer separately for males and females, by age. Titer levels were low at 14 days, but increased strongly by 28 days, and even more so at 42 days. Thus, immune response was not fully mature at four weeks of age. Titer levels of males and females were closely similar at all ages. On the basis of these results and others (data not shown) the experiments that follow were carried out on unsexed chicks.

Experiment 2

The purpose of this experiment was to obtain initial information on the time course of development of the immune response in light and heavy breed chicks. Seven consecutive trials were carried out, involving light breed chicks (Leghorn x New Hampshire), heavy breed chicks (White Rock 1, White Rock 2 and commercial broilers) and two antigens: E. coli vaccine (10^9 bacteria/kg bodyweight), and SRBC (10^8 cells/kg bodyweight). In trials 4 and 5 the number of plaque forming cells (PFC) was also determined. Table 2 shows titer levels by age and breed for the E. coli vaccinations, Table 3 gives similar information for the SRBC vaccinations as well as PFC data for trials 4 and 5.

With respect to E. coli antigen (Table 2), the data show some response by 1 week of age in the Leghorn x New Hampshire and White Rock 1 chicks, but

not in White Rock 2 chicks. By 11 days of age in the Leghorn x New Hampshire chicks, and by 10 days of age in White Rock 1 all chicks showed at least minimal antibody titers. In White Rock 2, however, even at 14 days of age, almost half of the birds did not respond to immunization. Following the initial appearance of antibody, there was a gradual increase in titer values continuing in each case until the end of the trial. Until about 22 days of age, immune response in the Leghorn x New Hampshire and White Rock 1 chicks increased in parallel, while that of White Rock 2 lagged behind. At 24 and 28 days there was a dramatic increase in immune response of the light breed chicks as compared to the heavy breed chicks, while by 32-35 days the gap between White Rock 1 and White Rock 2 appeared to be closing.

Immune response to SRBC (Table 3) was generally greater and developed earlier than to E. coli. Immune response in light and heavy breed chicks developed more or less in parallel until day 20. Thereafter, immune response of light breed chicks leveled off, or even declined, while that of heavy breed chicks continued to increase, reaching levels by day 35 that were considerably greater than those of the light breed chicks.

In the heavy breed chicks the number of PFC/ 10^6 spleen cells (Table 3) was initially low (day 6), but then increased rapidly, quickly reaching and maintaining high values. In the light breed chicks, PFC numbers were initially high (day 6), decreased greatly (days 10 and 13) and then increased again to high and stable levels. Since the experiment was not repeated it is not clear whether this is a true reflection of the biology of the system, or an experimental artifact. Aside from this there does not appear to be any clear difference in PFC numbers in light and heavy breeds. Because of the anomalous behavior of the light breed chicks, it is not possible to draw firm conclusions as to the relation between number of PFC and degree of immune response.

Experiment 3

In view of the differences between light and heavy breeds in the pattern of development of the immune response to *E. coli* and SRBC that was observed in Experiment 2, a further experiment was carried out to confirm these results. Groups of birds of each breed were immunized simultaneously at various ages with *E. coli* antigen (4×10^7 bacteria/chick from 6 to 21 days of age, 4×10^8 bacteria/chick, thereafter) and SRBC (0.2 cc. of a 20% SRBC suspension per chick). In addition, White Rock 2 chicks were also immunized with BSA (10 mg/kg bodyweight) in order to enable patterns of development of immune response to a variety of antigens to be compared within a breed.

Figure 1 shows $\log_2$ antibody titer values to *E. coli* vaccine. (White Rock 1 is identified as ANAK-80 in this figure and also in Figure 2). Once again at 24 days the light breed (Leghorn, in this case) shows a sharp increase in immune response as compared to the two heavy breeds. Also, White Rock 1 again shows precocious development relative to the light breed and White Rock 2. By day 12 all chicks responded in White Rock 1, but not until day 15 in the Leghorn and day 18 in White Rock 2. By day 42 all three breeds were at similar levels, but light breed titer values seemed to be at a plateau, while the heavy breed titer values were still increasing. Thus, these results confirm the more rapid early development of immune response in the light breed observed in Experiment 2, but suggest that mature response levels may be similar in the various breeds.

Figure 2 compares antibody titer to SRBC in the three stocks. Rate of development in light and heavy breeds was similar until about day 20-24. Thereafter the light breed titer levels plateaued, while heavy breed titer levels continued to increase until 5-6 weeks. A similar differential pattern of response was also observed in Experiment 2. Thus, in this case as well, the results of this experiment confirm the early similarity and late

differences in response to SRBC between light and heavy breeds, found in Experiment 2.

Figure 3 compares antibody response to E. coli, SRBC and BSA in White Rock 2 chicks. Immune response to SRBC developed earlier than to BSA or E. coli, but reached maximal values by week 5-6. In contrast, immune response to BSA and E. coli began later, but showed no indication of having reached maximal values by 6 weeks. Although the response to both BSA and E. coli began at about the same time (week 2), the rate of increase in response was greater for BSA than for E. coli.

Experiment 4

This experiment was carried out to determine whether the difference in developmental pattern of the immune response to E. coli between light and heavy breeds could be related to differences in macrophage activity (phagocytosis and killing by peritoneal macrophages). The experiment was carried out on Leghorn x New Hampshire and White Rock 2 chicks. Immune response of the two breeds to E. coli was also determined in separate samples of the chicks in order to confirm that the previously observed differences in developmental patterns were present.

Table 4 shows antibody titer to E. coli antigen (0.5×10^8 bacteria/chick) by age and breed. The high response of the light breed chicks at 4 weeks as compared to the heavy breed chicks was again observed. Similarly, titer levels of the light breed chicks again showed a plateau at 5 weeks, while titer levels of the heavy breed chicks continued to increase. Thus, these results fully confirm the previous results of Experiments 2 and 3.

Table 5 shows K-index values (see Methods) for phagocytosis and killing of E. coli by peritoneal macrophages, by breed and age. At weeks 3 and 4, peritoneal macrophages of light breed chicks were more active in phagocytosis and killing of E. coli than those of the heavy breed. This parallels the

period when immune response of light breed chicks exceeds that of the heavy breed chicks, and is suggestive of a possible relationship between the two phenomena. This point will be addressed further in the Discussion section.

Table 1. Antibody titer ($\log_2$) in White Rock chicks after immunization with E. coli vaccine by age and sex. Experiment 1.

Age	Males		Females	
	No.	Titer	No.	Titer
0	18	0.00	18	0.00
14	16	1.30	16	0.93
28	18	3.50	18	3.38
42	13	5.76	14	5.30

Table 2. Antibody titer ($\log_2$) after immunization by E. coli vaccine
by breed of chick and age at immunization. Experiment 2.

Leghorn x New Hampshire ¹ (Trial 1)		White Rock 1 ¹ (Trial 2)		White Rock 2 ² (Trial 3)	
Age	Titer	Age	Titer	Age	Titer
8	1.40	6	0.15	1	0.00
11	1.75	10	2.20	7	0.00
15	2.25	14	2.50	14	1.08
18	3.00	19	3.80	21	2.40
22	3.15	21	3.75	28	3.36
24	5.90	26	3.80	35	3.86
28	6.35	32	4.10	42	5.03

¹20 chicks per group. ²60 chicks per group.

Table 3. Antibody titer ($\log_2$) and PFC (per 10^6 spleen cells) after immunization with

SRBC by breed of chick and age at immunization (Experiment 2).

Leghorn x New Hampshire (Trial 4)			Commercial broiler (Trial 5)			White Rock 1 (Trial 6)			White Rock 2 (Trial 7)		
Age	Titer	PFC	Age	Titer	PFC	Age	Titer	PFC	Age	Titer	
Replicate 1 Replicate 2											
6	1.7	667	5	1.8	7	6	1.2	4	1	0.0	0.0
10	2.7	31	10	4.8	630	10	3.9	210	7	2.0	1.25
13	4.7	111	13	5.0	854	14	6.2	420	14	4.0	5.35
16	6.5	687	16	4.7	484	16	5.2	280	21	6.0	6.8
20	6.7	630				21	5.2	3100	28	6.5	6.2
25	5.3	618				28	5.9	1134	35	7.0	7.7
35	5.7	851				32	7.2	590			
						36	7.9	1089			

Table 4. Antibody titer ($\log_2$) after immunization with E. coli vaccine by breed and age at immunization (Experiment 4).

Age	Leghorn x New Hampshire	White Rock 2
7	0.00	0.00
14	0.00	0.00
21	3.64	2.75
28	5.24	3.76
35	4.93	4.45

Table 5. K-index values for phagocytosis and killing of E. coli
by peritoneal macrophages by breed and age

Age	Phagocytosis		Killing	
	Leghorn x New Hampshire	White Rock 2	Leghorn x New Hampshire	White Rock 2
14	0.0311	0.0315	0.0180	0.0069
21	0.0658	0.0410	0.0270	0.0200
28	0.0840	0.0565	0.0500	0.0310

Figure 1. Antibody titer (log₂) after immunization by E. coli vaccine by breed of chick and age at immunization. Experiment 3.

Figure 2. Antibody titer (log₂) after immunization by SRBC, by breed of chick and age at immunization. Experiment 3.

Figure 3. Antibody titer ($\log_2$) in White Rock chicks at various ages after immunization with E. coli vaccin, SRBC and BSA. Experiment 3.

D.2.2 Replicate A

Table 1 shows the arithmetic mean of the proportionate values for E. coli, NDV and Titer index, separately for high and low lines of Replicate A. In the parental population the high and low line means represent the founding families of the high and low lines; in the S-1 and subsequent generations, the high and low line means represent the total set of sire families within each line. Differences between the high and low lines, and their significance (by t-test) are also shown. Differences between high and low lines were maximal for the parental generation, as can be expected since this represents a phenotypic selection of highs and lows. However, the difference between high and low lines in the S-1 were remarkably high: greater than the original parental difference for E. coli; and 66% and 81% of the original parental difference for NDV and Titer index, respectively. That is, realized heritabilities were 1.55, 0.66 and 0.81, respectively. The large E. coli difference in the S-1 generation may be due to a correlated effect of the NDV selection in the parental generation. In the S-2 generation, there is little information on E. coli or index values, since most families did not have values for E. coli titer. However, the difference between lines for NDV was considerably lower than in the S-1 generation.

Table 2 shows the sire family means ranked from highest to lowest over both lines but separately for high and low lines, by generation of selection. This is done in order to show the degree of separation and overlap between the high and low lines. In the parental generation, separation was complete for index values, which is expected, of course, since this was the criterion of selection. Separation was also complete for NDV titers, but there was considerable overlap for E. coli titers. Thus, it appears

that NDV titer was the primary source of variation in index values in the parental generation. In the S-1 generation, separation of lines was virtually complete for titer index values. There was also relatively little overlap for E. coli and NDV values, with the exception of one very low family (not the same) in the high line for both E. coli and NDV. With these exceptions, the highest values for E. coli and NDV were found in the high line and the lowest values in the low line. In the S-2 generation, little can be said about E. coli and Titer index, because of the very few families with values for E. coli titer. Still it is suggestive that the two highest families were in the high line. But again, the very lowest family was also derived from the high line. For NDV, separation was very marked, except that the family with the very highest titer came from the low line.

From consideration of Table 1 and Table 2 it is clear that Titer index selection had a marked effect on relative titer values of the two lines for both antigens and for Titer index as well. Table 3 shows actual mean $\log_2$ titer values for the two lines by antigen and generation of selection. Relative absolute antibody concentration of high line as compared to low line are also shown. In the selection generation (S-1 and S-2) antibody concentration in the high line was about one-third greater than in the low line for both E. coli and NDV antigen. This seems an appreciable amount, and one that might have practical consequences in post-vaccination disease resistance.

Figure 1 shows the pedigree relations of the males in the S-3 generation before selection. In the high line, four males were selected in the parental generation and all of them still had descendant sire families represented in the S-3 generation, although one male (#10) contributed a disproportionate number (4 out of 10). In the low line, only two sires were selected in the

parental generation, and of these, one sire was represented by eight, the other by two descendant families in the S-3 generation. Thus, in the low line there was a considerable reduction in number of "bloodlines" during the selection process, while the high line appears to be maintaining a more diverse genetic base.

Table 4 shows the mean proportionate value of the selected sire families in the parental, S-1 and S-2 generations, for the two antigens and the Titer index. The selection differential (i.e., the difference between selected sire families and overall mean of sire family values for a particular generation) and line are also shown, as well as the cumulated selection differential. Values for the selection differential for E. coli in generation S-2 could not be calculated, since only three sire families were evaluated for this antigen in the high line, and the same number in the low line. Consideration of Table 4 shows that the bulk of the selection was achieved in the parental generation. In the S-1 generation there was a slight additional selection in the high line, but a slight contrary selection in the low line (i.e., the selected families were slightly above average in E. coli and NDV titer!). In the S-2 generation, E. coli titers were not available for most families, so that there was probably no net selection differential for this trait. For NDV, however, selection differential in the high line was contrary to the direction of selection (i.e., the selected sire families had a slightly lower titer than the overall generation mean of the high line), while in the low line, selection differentials were in the direction of selection. The total cumulative selection differential between lines was virtually unchanged in the S-1 and S-2 generations as compared to the parental generation. Thus, these results show that on the whole family selection in the S-1 and S-2 generations did not make any further contribution to differentiation between the lines,

beyond that achieved in the parental generation.

Table 5 shows a comparison of the selection lines to two commercial White Rock broiler strain lines, with respect to NDV and E. coli titer. The expected difference between high and low lines in E. coli and NDV titer was found in the test. With respect to NDV, the high line value fell between the values for the two commercial strains, while low line value was the least of all lines. With respect to E. coli, high line value was greater than either of the commercial lines, while low line value was just a trifle greater than the lower of the two commercial lines. These data suggest that the low line is indeed a low responder, and give some indication, at least, of true superiority for the high line.

Table 6 shows overall mortality for the unselected S-2 generation chicks of the high and low line, and also mortality as a result of incomplete inactivation of the E. coli vaccine used for test purposes. The differences between the two lines in overall mortality and in mortality on exposure to the incompletely inactivated E. coli vaccine was highly significant in both cases, and in favor of the high line. Incomplete inactivation of E. coli vaccine occurred twice in the S-3 generation of chicks as well. Results are shown in Table 7. In both cases, mortality of high line chicks was significantly less than that of low line chicks ($p < 0.01$, $p < 0.05$ for first and second hatches, respectively).

D.2.2 Replicate B

The response to selection in these lines was very powerful. In order to confirm this response, a similar selection routine was carried out for one generation only in a second White Rock population. Table 8 shows the proportionate values for E. coli, NDV and Titer Index of the parental and S-1 generations. High and low lines differed significantly in the S-1 generation, and realized heritabilities of 0.80, 0.53 and 0.59 were

obtained for E. coli, NDV and Titer Index, respectively.

Table 9 shows individual sire family ranking for Parental and S-1 generation of the Replicate B. In this case, as well, a clear separation of the lines can be discerned in the S-1 generation, with the extreme high individuals for both E. coli, NDV and titer index coming from the high line. The extreme low individual again there was one very low individual for both E. coli and NDV in the high line as well.

Table 10 shows absolute $\log_2$ titer values for the high and low lines of the second replicate by antigen, and relative antigen concentration of high line as compared to low line. Absolute high line titer values were 60% greater for E. coli and 99% greater for NDV.

Thus, the results of the second replicate fully confirm the essential results of the main selection line with respect to the very high realized heritability of family selection for high and low titer values to NDV and E. coli; and, the ability of one generation of selection to produce good separation between family mean values, and a significant difference in absolute titer values, between high and low lines.

Figure 1. Pedigree relations of males in the S-3 generation before selection, separately for high and low lines, Replicate A.

Table 1. Arithmetic mean of sire family proportionate values for high and low lines of Replicate A, by generation of selection.

Generation	E. coli		NDV		Titer Index	
	No.	Value	No.	Value	No.	Value
Parental						
High	10	1.051	10	1.163	10	1.104
Low	8	0.930	8	0.807	8	0.863
Difference		0.121*		0.356**		0.274**
S-1						
High	7	1.104	7	1.080	7	1.096
Low	7	0.916	7	0.844	7	0.870
Difference		0.188*		0.236*		0.226**
S-2						
High	4	1.250	10	1.091	2	1.170
Low	3	0.753	9	0.962	3	0.820
Difference		0.497 ^{NS}		0.129*		0.350*

Parental						S-1						S-2					
E. coli		NDV		Titer Index		E. coli		NDV		Titer Index		E. coli		NDV		Titer Index	
High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
1	1	1.5		1		1		1		1		1		1		1	
2		1.5		2.5		2		2		2		2		2		2.5	
3		3.5		2.5		3		3		3		3		3		4	
4.5		35.		4		4		4.5		4		4		4		5	
4.5		5		5		5		6		5		5		5			
	6	7.5		6		6		7		6		6		6			
7.5		7.5		7		8		8		8		7		7.5		7.5	
7.5		7.5		8		8		10		10		8		9.5		9.5	
	9	7.5		9		9		10		9		11		11			
10	11	10		10		10.5		12.5		10		12		12			
	11			11		10.5		12.5		11				13			
12				12		12		14		12		14					
	13.5			13		13		15		13				15			
	13.5			14		15								16.5			
15.5	15.5			15		15								16.5			
16				16		15								18			
	17			18										19			

Table 3. Mean $\log_2$ titer values and relative antibody concentration¹ for the selection lines by antigen and generation (Replicate A).

Generation	E. coli	NDV
Parental		
High	2.06	2.83
Low	1.59	1.53
Relative conc.	1.38	2.46
S-1		
High	1.64	5.66
Low	1.43	5.25
Relative conc.	1.15	1.32
S-2		
High	1.66	5.58
Low	1.12	5.12
Relative conc.	1.45	1.37

¹Relative concentration = $\text{antilog}_2 \text{ High} / \text{antilog}_2 \text{ Low}$

Table 4. Mean proportionate value of selected sire families, selection differential (S.D.) and cumulative selection differential (C.S.D.) by antigen and generation of selection. Replicate A.

Generation	E. coli			NDV			Titer Index		
	Selected families	S.D.	C.S.D.	Selected families	S.D.	C.S.D.	Selected families	S.D.	C.S.D.
Parental									
High	1.051	+0.051	+0.051	1.163	+0.163	+0.163	1.104	+0.104	+0.104
Low	0.930	-0.070	-0.070	0.807	-0.193	-0.193	0.863	-0.137	-0.137
Total			0.121			0.356			0.241
S-1									
High	1.118	+0.014	+0.065	1.088	+0.008	+0.171	1.110	+0.004	+0.108
Low	0.983	+0.067	-0.003	0.853	+0.009	-0.184	0.920	+0.005	-0.132
Total			0.126			0.355			0.240
S-2 ¹									
High				1.050	-0.041	+0.130			
Low				0.912	-0.050	-0.234			
Total						0.364			

¹Values are not given for E. coli and Titer Index, since E. coli titers were available for three high line and three low line families only.

Table 5. Comparison of antibody titer values ($\log_2$) of selection lines¹
and of two commercial White Rock lines. Replicate A.

Line	E. coli		NDV	
	No.	Titer	No.	Titer
61	45	4.26	25	1.92
41	41	3.57	25	1.72
High	24	3.83	27	2.50
Low	29	2.76	23	1.74

¹
S-4 chicks.

Table 6. Overall mortality and mortality on exposure to E. coli in selection lines (S-2). Replicate A.

Line	Overall mortality		E. coli	
	Total chicks	% Mortality	Total chicks	% Mortality
High	437	19	98	3
Low	290	29	64	11

Table 7. Mortality on exposure to E. coli in selection lines (S-3). Replicate A.

Line	Hatch 1		VV	Hatch 2	
	Total chicks	% Mortality		Total chicks	% Mortality
High	67	6		97	10
Low	61	18		78	17

Table 8. Arithmetic mean of sire family proportionate values for high and low lines by generation of selection. Replicate B.

Generation	E. coli		NDV		Titer Index	
	No.	Value	No.	Value	No.	Value
Parental						
High	8	1.26	9	1.11	8	1.21
Low	9	0.60	9	0.81	9	0.70
Difference		0.66**		0.30**		0.51**
S-1						
High	13	1.20	12	1.06	12	1.14
Low	12	0.77	12	0.90	12	0.84
Difference		0.53**		0.16*		0.30**

Table 9. Sire family ranking, by antigen and index values by selection line and generation of selection. Replicate B.

[illegible]

Table 10. Mean $\log_2$ titer values and relative antibody concentration¹
for the selection lines by antigen and generation (Replicate B).

Generation	E. coli	NDV
Parental		
High	1.54	3.52
Low	0.75	2.58
Relative concentration	2.20	2.56
S-1		
High	1.31	3.84
Low	0.84	3.15
Relative concentration	1.60	1.99

¹Relative concentration = $\text{antilog}_2 \text{ High} / \text{antilog}_2 \text{ Low}$

D.2.3 Discussion and Conclusions

The results of this study show clear indications of differences between poultry populations in the pattern of development of the immune response to specific antigens. In particular, light breeds (Leghorn and Leghorn x New Hampshire) showed rapid development of response to *E. coli* vaccine, that reached an early plateau, while heavy breeds showed a slower initial development that continued, however, to increase steadily within the time period of the experiments, eventually equalling and even exceeding the light breed response. With respect to sheep red blood cells, patterns of development differed from the above. In all populations response to SRBC developed earlier and was more powerful than the response to *E. coli*. In this case, however, early response of light and heavy breeds developed in parallel, but then the response of the light breeds leveled off, while response of the heavy breeds continued to increase for another week or two before levelling off in turn. Studies at a cellular level showed that macrophage activity against *E. coli* (phagocytosis and killing) was greater in the light breeds at the time when their immune response was also greater than that of the heavy breeds. This suggests that in these populations, increased immune response may be associated with increased macrophage activity. This is in contrast to the observations on the high and low immune response lines in mice developed by Biozzi (Doria et al., 1978; Adorini and Doria, 1981; Biozzi et al., 1979), in which macrophage activity of the high line was less than that of the low line.

The results of the selection experiments show that a powerful response is obtained to family selection for high and low early immune response to disease antigens. In two experiments realized heritabilities for immune response were over 0.50 for both antigens. Comparison of the selection lines to two commercial lines, gave clear indications of low immune

competence for the low line, but did not show a clear superiority for the high line. It is possible, however, that a few more generations of selection might change this picture. A very exciting preliminary result of this study is the clear and consistent superiority of the high line as compared to the low line with respect to overall mortality, and specific mortality when challenged with *E. coli*. This is in contrast to the results of studies on the Biozzi high and low lines (Biozzi et al., 1979), in which the low line generally showed greater natural and post-vaccination resistance to bacteria. This was explained as due to the greater macrophage activity in the low line (Biozzi et al., 1979). In our studies, macrophage activity was greater in light breeds during the period of their greater immune response. If a similar condition obtains in our high and low selection lines, this could also explain the greater overall and specific resistance of the high line.

In conclusion, the results of this study show that differences in rate of development of immune response to specific disease antigens are present both between and within commercial poultry populations, and that these differences respond strongly to selection. Initial observations suggest that differences in specific immune response may be related to differences in specific disease resistance. Clearly these results must be confirmed and extended, but they point to the exciting possibility of testing for early immune response to specific disease antigens as a means of genetic selection for improved immune competence and resistance with respect to these disease agents.

References

- Adorini, L. and G. Doria. 1981. *Eur. J. Immunol.* 11: 984-989.
- Biozzi, G., et al., 1979. *Current Topics in Microbiology and Immunology* 85: 31-98.

Doria, G., B. Agarossi and G. Biozzi. 1978. Immunology 34: 999-1005.

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E. Evaluation of the research achievement with respect to the original research proposal.

1. It was determined by strain comparisons and within population studies that genetic variation in the rate of development of immune competence exists both between poultry populations and within poultry populations.
2. It was demonstrated by selection experiments that this variation has a genetic basis.
3. It was shown that the genetic variation is related to specific antigens.
4. Initial studies were carried out relating between strain differences in immune response (to SRBC) to variation in competence at the cellular level (macrophage activity).
5. Due to technical and space limitations it did not prove possible within the framework of this proposal to relate genetic variation in early response to variation in mature responsiveness or to B-locus alleles.

F. Description and results of cooperation

Prof. Nordskog visited Israel in the first year of the project. His participation was very helpful in defining our goals and in designing the selection program. Prof. Heller spent two weeks at Ames in the second year of the project and received training and reagents for identification of B₂ locus alleles. Prof. Soller visited Ames in the third year of the project to discuss the results and implications of the research with Prof. Nordskog.

G. Theses: none

H. Benefit to Agriculture

1. The existence of genetic differences in rate of development of immune competence to specific disease antigens, within heavy breed (broiler) populations, has been established, and a strong response to selection has been demonstrated. Initial results suggest that high response is associated with increased resistance to the disease.
2. These results if confirmed and extended point the way to a practical means of selecting directly for specific disease resistance and immune competence in poultry.
3. The approach pioneered here, may have broad general applicability to other agriculture animal species.

I. Inventions: none

J. Publications:

1. Differences between light and heavy breeds of poultry in pattern of development of immunological maturity with respect to E. coli vaccine and Sheep red blood cells (in preparation).

2. Two-way selection for early immune response to the disease antigens, *E. coli* and NDV, in poultry (in preparation).
3. Early immune response to *E. coli* and NDV vaccine in poultry in relation to resistance to challenge (in preparation).