STUDIES ON GENETIC RESISTANCE TO PULLORUM DISEASE IN CHICKS

By

Yoshio HACHINOHE, Hitoshi MIKAMI, Yoshio TSUTSUMI
Ikuo OKADA and Hiroshi SHIMIZU

(Department of Animal Science, Faculty of Agriculture,
Hokkaido University, Sapporo, Japan)

Ichiro TAKAYASU

(Faculty of Agriculture, Hirosaki University, Hirosaki, Japan)

Pullorum disease is caused by the bacterium, *Salmonella pullorum*. Affected chicks show symptoms within a few days after hatching and the peak of mortality is usually passed within two weeks. Since the fact that the causative bacterium is transmitted by hens through some of their eggs to the chicks was found, it has become standard practice in poultry production to cull the hens that carried the bacteria by the method of agglutination test for *S. pullorum*.

Evidence that chicks have various genetic abilities of resistance to the pullorum disease has been reported by several investigators. HUTT and SCHOLES (6) mentioned that Leghorns were more resistant than heavy breeds, and ROBERTS and CARD (14) claimed that natural resistance was enhanced by selective breeding in White Leghorns. DEVOLT et al. (3) also obtained the similar results using Rhode Island Reds.

Several investigations have been performed to search for some detectable physiological differences between chicks' resistance to pullorum disease and the susceptibility to it. SEVERENS et al. (16) attributed the differences in susceptibility to the proportion of the number of lymphocytes during the first few days after hatching. RAM and HUTT (13) demonstrated that genetic resistance to pullorum disease was associated with superior control of the thermoregulatory mechanism. Recently HUTT and CRAWFORD (5) practised high body temperature selection over two generations. The chicks selected for the high body temperature were consistently more resistant to experimental inoculation with *S. pullorum* than those selected for low temperatures.

The present experiments were designed to confirm the differences in susceptibility to pullorum disease among breeds and strains in chicks. Further-

[Journ. Facul. Agr., Hokkaido Univ., Sapporo, Vol. 55, Pt. 2, 1967]

more, the susceptibility to this disease was investigated in relation to sex, blood group, number of leucocytes and body temperature.

MATERIALS AND METHODS

Stock

Chicks of five different breeds, White Leghorns, White Plymouth Rocks, New Hampshires, Hinais and Onagas (Long Tailed Fowls), and DeKalb strain were used in the present experiments. White Leghorns consisted of five strains, which were designated B, F, G, N and S. The N strain was kept at the Experimental Farm of Hokkaido University ever since it was introduced in this farm from the Takikawa Animal Husbandry Experiment Station in 1957. The S strain was introduced in this station from the Shintoku Animal Husbandry Experiment Station in 1962 and the B strain from Omiya Livestock Breeding Station in 1956. The G and F strains were obtained from the Omiya Livestock Breeding Station. The flock of White Plymouth Rocks was introduced in the Experimental Farm of Hokkaido University from United States of America in 1960 and 1961. New Hampshires were obtained from a private farm in Tomakomai, and Hinais and Onagas (which are Japanese native breeds) were obtained from bird-fanciers in the Tohoku district. The DeKalb strain was recently imported into Japan from United States of America.

Management

In order to avoid the influence of differences in hatching time, chicks hatched within a 24-hour period were chosen for experiments. At 24 hours after hatching, they were removed from incubator to the specially designed brooder in which the temperature was regulated by thermostat. The brooder temperature was maintained at 35°C. and light was constantly provided. All brooders and equipment were thoroughly cleaned and sterilized by steam and formaldehyde gas before each experiment. The formula feed used did not contain any medicine. In order to check infection with *S. pullorum*, the fecal culture of all chicks used for the experiments, were inspected. No Salmonella was isolated from the chicks before inoculations. The flocks in the Experimental Farm of Hokkaido University have been shown by agglutination tests to be free from *S. pullorum* since 1962.

Inoculation

One strain of *S. pullorum* which originated from the strain of Dr. MIURA's laboratory of Hokkaido University was used in the present experiments. Livers of chicks which died after inoculation were preserved at −20°C. and the cultures used for inoculation were reisolated from them as occasion demand. Chicks

were inoculated orally at 48 hours after hatching with 0.1 ml of a 24-hour YCC broth culture. The number of bacterial cells in this culture determined by direct count on agar and ranged from 3×10^8 to 2.1×10^9 per ml from experiment to experiment.

Diagnosis

The number of dead chicks was recorded every morning and evening until 21 days after inoculation. The recording ended when all chicks belonging to one of the subgroups died. The culture of every dead chick was examined. Portions of heart, spleen and liver removed aseptically were cut into pieces and placed on brilliant green agar and incubated for 48 hours at 37°C. In addition to observation of the characteristic colonies of *S. pullorum*, confirmation was made with appropriate antiserum.

Techniques for counting leucocytes

Blood samples obtained from wing vein were diluted 1 : 10 with NATT and HERRICK's diluting solution (10) using pipettes for red blood cell counting. They were shaken for one minute. All direct counts were made with Thoma's haemocytometers.

Techniques for blood typing

Blood typing and the nomenclature used in the present experiments was performed by the methods of MATSUMOTO and OKADA (9) and OKADA, TAKAGI and MATSUMOTO (11).

Recording body temperature

One hour prior to recording body temperatures, chicks were transferred to a climatic chamber in which the temperature and relative humidity were maintained at 30°C. and 60 percent, respectively. In this chamer body temperatures were measured by a thermocouple held one minute in the cloaca and recorded to the nearest 0.01°C Insertion into the cloaca was done to a uniform depth of 1.5 cm and recordings were made from 1.00 to 4.00 p.m. each day to avoid the influence of diurnal variation, which is an important source of error according to LAMOREUX and HUTT (8).

RESULTS

Breed differences in susceptibility

Relative susceptibility among the five breeds, White Leghorns, White Plymouth Rocks, New Hampshires, Hinais and Onagas were examined by inoculation with *S. pullorum* (Table 1). To test for statistical singnificance, the χ^2 test was performed. The White Leghorns were significantly more resistant

TABLE 1. Differential susceptibility of breeds to inoculation with 6.5×10^7 *S. pullorum*.

Breed	Chicks inoculated	Mortality		Significance of difference from			
	No.	No.	Percent	White Leghorns	White Plymouth Rocks	New Hampshires	Hinais
White Leghorns	12	0	0				
White Plymouth Rocks	12	2	16.7	—			
New Hampshires	12	8	66.7	**	*		
Hinais	12	5	41.7	*	—	—	
Onagas	10	7	70.0	**	*	—	—

—=Not significant.
*=Significant at 5% level.
**=Significant at 1% level.

than the New Hampshires, Hinais and Onagas, but the difference between the White Leghorns and the White Plymouth Rocks was not significant.

Strain differences of White Leghorns in susceptibility

The S, N and B strain chicks were used first to determine the relative susceptibilities in strains. The N strain was more susceptible than the S and B strains (Table 2). In the second experiment, the S, N, G and F strain chicks

TABLE 2. Differential susceptbility of strains of White Leghorns to inoculation with 6.5×10^7 *S. pullorum*.

Strain	Chicks inoculated	Mortality		Significance of difference from	
	No.	No.	Percent	S	N
S	12	3	25.0		
N	12	12	100.0	**	
B	12	7	58.3	—	*

—=Not significant.
*=Significant at 5% level.
**=Significant at 1% level.

were used and these results are summarised in Table 3. The S strain was significantly more resistant than the N and F strains. Both the experiments showed that the S strain was more resistant than the N strain. In order to confirm this finding, comparative experiments between these two strains were repeated six times, and their results are given in Table 4. Since the number

TABLE 3. Differential susceptibility of strains of White Leghorns to inoculation with 3.0×10^7 *S. pullorum*.

Strain	Chicks inoculated	Mortality		Significance of difference from		
	No.	No.	Percent	S	N	G
S	12	1	8.3			
N	12	6	50.0	*		
G	12	4	33.3	—	—	
F	12	6	50.0	*	—	—

—=Not significant.
*=Significant at 5% level.

TABLE 4. Mortality of the S and N strains to inoculation with *S. pullorum*.

Arcsin $\sqrt{\text{Proportion}}$

Strain	Replication						
	1	2	3	4	5	6	Mean
	Inoculated dose						
	6.5×10^7	3.0×10^7	1.0×10^8	1.8×10^8	1.5×10^8	1.2×10^8	
S	30.0 (24)	16.7 (12)	16.7 (12)	49.8 (12)	11.8 (24)	30.0 (12)	25.9 (96)
N	90.0 (12)	45.0 (24)	41.0 (72)	90.0 (12)	40.2 (12)	90.0 (8)	66.0 (140)

Numbers of chicks inoculated are shown in parentheses.

Analysis of variance of mortality

Source of variation	Degrees of freedom	Sum of squares	Mean square
Strains	1	4846.51	4846.51**
Replications	5	3767,11	753.42*
Error	5	659.62	131.92

*=Significant at 5% level.
**=Significant at 1% level.

of inoculated chicks varied with the experiments, statistical analyses were made in terms of proportions, which were transformed to the arcsin proportion (SNEDECOR, 17). The S strain again showed significantly higher resistance than the N strain though the differences among the replications were also

significant. Therefore, the S and N strains were used as the resistant and susceptible strains, respectively, in further experiments.

Comparison was carried out of the susceptibility of the commercial chicks of the DeKalb strain with that of the S strain chicks, because we were much interested in the resistance to disease of American commercial chicks. The results are shown in Table 5. The commercial chicks were more rsistant than the S strain which was the most resistant strain in the White Leghorns used in the present study.

TABLE 5. Comparison of susceptibility to inoculation with 2.1×10^8 *S. pullorum* of the S strain with the commercial chicks of the DeKalb strain.

Strain	Chicks inoculated	Mortality		Significance of difference (χ^2)
	No.	No.	Percent	
S	14	13	92.9	22.91**
Commercial Chicks	20	2	10.0	

**=Significant at 1% level.

Inheritance of susceptibility

The S strain was mated with the N strain and their F_1 generations were mated back to both of the parent's strains to investigate inheritance of susceptibility to pullorum disease. The S and N strain chicks and their F_1 chicks were inoculated first (Table 6). The F_1 chicks were fully as resistant as the S strain chicks. In the following two experiments, the S and N strain chicks and the chicks from reciprocal crosses between the S and N strain. and from

TABLE 6. Differential susceptibility of the S sirain, the N strain and their F_1 generation to inoculation with 1.8×10^8 *S. pullorum*.

Strain	Chicks inoculated	Mortality		Significance of difference from	
	No.	No.	Percent	S	N
S	12	7	58.3		
N	12	12	100.0	*	
F_1	12	6	50.0	—	**

—=Not significant.
*=Significant at 5% level.
**=Significant at 1% level.

four different backcrosses were inoculated (Table 7, 8). The F_1 chicks and the S strain chicks again showed similar resistance and the difference between the F_1 and N strain chicks was statistically significant. There was no difference between the reciprocal crosses. The chicks from four different backcrosses

TABLE 7. Differential susceptibility of the *S* strain and N strain, their F_1 and backcrosses to inoculation with 1.5×10^8 *S. pullorum*.

Strain	Chicks inoculated	Mortality		Significance of difference from						
	No.	No.	Percent	S	N	$F_1^{a)}$	$F_1^{b)}$	$F_1^{a)}\times S$	$F_1^{b)}\times S$	$F_1^{a)}\times N$
S	12	1	8.3							
N	12	5	41.7	—						
$F_1^{a)}$	12	0	0	—	*					
$F_1^{b)}$	12	0	0	—	*	—				
$F_1^{a)}\times S$	12	2	16.7	—	—	—	—			
$F_1^{b)}\times S$	12	2	16.7	—	—	—	—	—		
$F_1^{a)}\times N$	12	0	0	—	*	—	—	—	—	
$F_1^{b)}\times N$	12	1	8.3	—	—	—	—	—	—	—

—=Not significant.
*=Significant at 5% level.
$F_1^{a)}$=The N strain (♀♀)×the S strain (♂♂).
$F_1^{b)}$=The S strain (♀♀)×the N strain (♂♂).

TABLE 8. Differential susceptibility of the S strain and N strain, their F_1 and backcrosses to inoculation with 1.2×10^8 *S. pullorum*.

Strain	Chicks inoculated	Mortality		Significance of difference from						
	No.	No.	Percent	S	N	$F_1^{a)}$	$F_1^{b)}$	$F_1^{a)}\times S$	$F_1^{b)}\times S$	$F_1^{a)}\times N$
S	12	3	25.0							
N	8	8	100.0	**						
$F_1^{a)}$	12	5	41.7	—	**					
$F_1^{b)}$	12	5	41.7	—	**	—				
$F_1^{a)}\times S$	12	1	8.3	—	**	—	—			
$F_1^{b)}\times S$	12	1	8.3	—	**	—	—	—		
$F_1^{a)}\times N$	12	3	25.0	—	**	—	—	—	—	
$F_1^{b)}\times N$	12	4	33.3	—	**	—	—	—	—	—

—=Not significant.
*=Significant at 5% level.
**=Significant at 1% level.
$F_1^{a)}$=The N strain (♀♀)×the S strain (♂♂).
$F_1^{b)}$=The S strain (♀♀)×the N strain (♂♂).

were as resistant as the S strain and F_1 chicks, and there were no differences among these four back-crosses. The differences between the S and N strain, and between the N strain and three of these backcrosses were, however, not statistically significant in the experiment of which results are given in Table 7.

The commercial chicks of the DeKalb strain were shown to be highly resistant to pullorum disease in the previous experiment (Table 5). Then it was investigated whether this resistance came from either one or both of their parent's lines. It is clearly seen in Table 9 that the resistance of the commercial

TABLE 9. Differential susceptibility of the commercial chicks and their both parent lines chicks to inoculation with 2.0×10^8 *S. pullorum*.

Strain	Chicks inoculated	Mortality		Significance of difference from	
	No.	No.	Percent	Maternal line	Paternal line
Maternal line	28	2	7.1		
Paternal line	28	10	35.7	*	
Commercial line	28	0	0	—	**

—=Not significant.
*=Significant at 5% level.
**=Significant at 1% level.

chicks was inherited from the maternal line. The maternal line chicks were as resistant as the commercial chicks, but the paternal line chicks were significantly more susceptible than the commercial chicks.

Sex difference in susceptibility

There may be sex differences in susceptibility to pullorum disease. Therefore, to detect the difference of mortality between sexes, live and dead chicks were examined. The 63 survivors of 93 chicks which had been inoculated were sexed by laparotomy. Thirty five chicks were male and 28 chicks were female. The value of χ^2 (0.29) shows that the difference was not significant. In another experiment, 142 chicks were inoculated and 32 dead chicks were sexed. Fifteen of them were male and 17 were female. The difference was also not significant ($\chi^2=0.13$). Thus, it is clear that there was no difference between the sexes. Therefore, the sex classification was omitted in following experiments.

Relationship between blood groups and susceptibility

It has been recognized that the B locus determining blood groups is con-

nected with fitness. Therefore, relationships between two allels, B^A and B^C of the B locus, and susceptibility to pullorum disease were investigated. The N strain was used in these experiments, and mating was designed to make comparisons between the homozygotes, B^AB^A and B^CB^C, and the heterozygote, B^AB^C. Twenty four homozygous chicks and the same number of heterozygous chicks were inoculated in the first experiment. As shown in Table 10, the

TABLE 10. Susceptibility of chicks classified by the B locus genotypes to inoculation with *S. pullorum*.

Experiment	Inoculated dose	Genotype	Chicks inoculated (No.)	Mortality No.	Mortality (Percent)	Significance of difference (χ^2)
1	5.0×10^7	Homozygote (B^AB^A, B^CB^C)	24	1	4.2	6.70**
		Heterozygote (B^AB^C)	24	8	33.3	
2	1.0×10^8	Homozygote (B^AB^A)	12	3	25.0	0.07
		(B^CB^C)	12	4	33.3	
		Heterozygote (B^AB^C)	12	3	25.0	

**=Significant at 1% level.

homozygous chicks were more resistant than the heterozygous chicks. In the following experiment, the homozygous chicks were divided into the B^AB^A group and B^CB^C group, and each of the 12 chicks of these two homozygous groups and of the heterozygous group were inoculated. The differences among them were not significant. Thus no conclusion could be drawn from these two experiments.

Relationship between total leucocyte number and susceptibility

Twenty chicks of each the S and N strain, and 14 chicks of their F_1 generation were used. Leucocytes were counted at each the 3, 6, 9, 12, 15, 20 and 25th day after hatching. As presented in Figure 1 and Table 11, the average leucocyte numbers of the three groups increased steadily untill the 25th day after hatching. The S strain chicks had significantly higher leucocyte numbers than those of the N strain chicks except at the 15 and 20th day after hatching. The differences between them at the 15 and 20th day were not significant. The leucocyte numbers of F_1 chicks were significantly lower than that of the S strain chicks at the 6, 9 and 12th day after hatching, but at the 20 and 25th day after hatching the F_1 chicks had the highest number and the difference between the F_1 and N strain chicks at the 25th after hatching

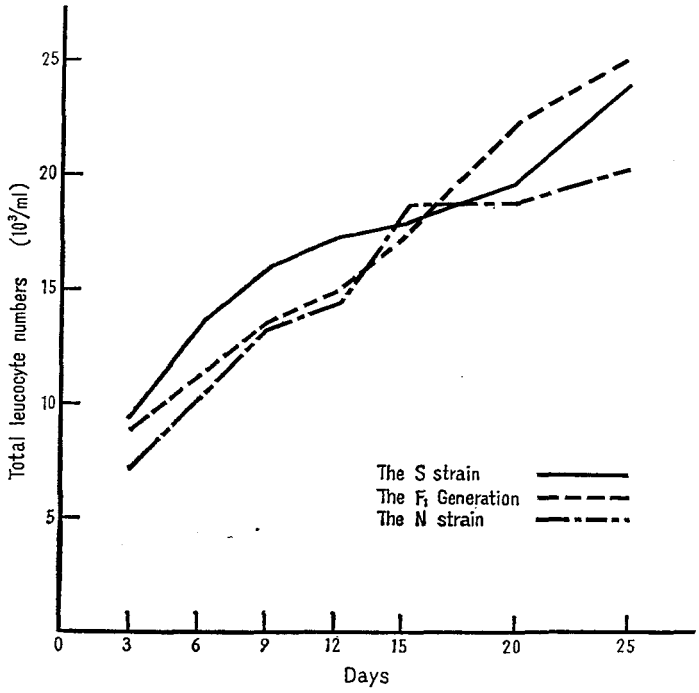

Fig. 1. Average total leucocyte numbers of the S strain, the N strain and their F_1 generation from 3 to 25 days afer hatching.

TABLE 11. Difference among average total leucocyte numbers of the S strain, the N strain and their F_1 generation.

Age of chicken (days)	Average total leucocyte numbers			Difference		
	S	N	F_1	S−N	S−F_1	F_1−N
3	9.55	7.29	8.95	+2.26**	+0.60	+1.66
6	13.94	10.14	11.29	+3.80**	+2.65*	+1.15
9	16.09	13.24	13.38	+2.85*	+2.71*	+0.14
12	17.43	14.56	14.96	+2.87**	+2.47*	+0.40
15	17.70	18.91	17.48	−1.21	+0.22	−1.43
20	19.78	18.94	22.48	+0.84	−2.70	+3.54
25	24.25	20.35	25.18	+3.90**	−0.93	+4.83**

*=Significant at 5% level.
**=Significant at 1% level.

was significant.

Since the average leucocyte numbers of the S strain during the first 15 days, which seemed to be the most important period, were significantly higher not only than those of the N strain, but also than those of the F_1 generation which showed resistance similar to the S strain, it was impossible to conclude that total leucocyte numbers were related to susceptibility to pullorum disease.

Relationship between body temperature and susceptibility

To ascertain whether there are differences among families in average body temperature during the first 3 days after hathching or not, family averages were determined for 2 sire families and 23 dam families. comprising 138 chicks of the N strain of White Leghorns, and for 2 sire families and 12 dam families, comprising 57 chicks of White Plymouth Rocks. The average number of chicks per dam was about 5.6. Each chick's body temperature was recorded at the first and third day after hatching. The average body temperatures of the White Leghorn and White Plymouth Rock chicks were 39.25° and 39.80°C the first, and 40.35° and 40.45°C. at the third day after hatching, respectively. Thus the White Leghorn chicks raised their temperatures more quicky than did the White Plymouth Rock chicks. The analyses of variance showed that there were significant differences between two breeds, among the four sire

TABLE 12. Analysis of variance for body temperature at the first day after hatching.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between breeds	1	12.160	12.160**
Among sires within breeds	4	5.109	1.277**
Among dams within sires	33	8.347	0.253**
Within dams	156	12.164	0.078

**=Significant at 1% level.

TABLE 13. Analysis of variance for body temperature at the third day after hatching.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between breeds	1	0.018	0.018
Among sires within breeds	4	1.423	0.356
Among dams within sires	33	6.292	0.191
Within dams	156	26.175	0.168

families, and also among the 22 dam families at the first day after hatching, but that there were no significant differences among them at the third day after hatching (Table 12, 13).

Then to determine whether these differences the first day after hatching were related to differences in susceptibility, body temperatures of 24 S strain and 12 N strain chicks were recorded at the first day after hatching and they were inoculated the following day. The results of this experiment are shown in Table 14. The average body temperatures of 18 live and 6 dead chicks of the S strain were 40.02° and 39.89°C., respectively, and this difference was

TABLE 14. Differences of body temperatures, measured at the first day after hatching, survived and dead chicks.

Strain	Chicks inoculated	Mortality		Average body temperature (°C)			Difference
	No.	No.	Percent	Survived (A)	Dead (B)	Total	(A)−(B)
S	24	6	25.0	40.02	39.89	39.98	0.13
N	12	12	100.0	—	39.68	39.68	—
					Difference	0.30	

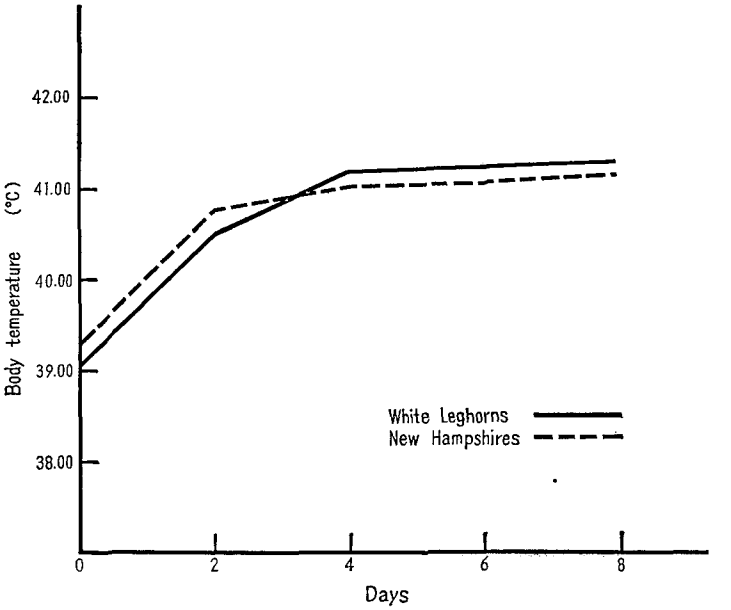

Fig. 2. Body temperature of the White Leghorns and the New Hampshires from 0 to 8 days after hatching.

not significant. Since all chicks of the N strain died, the comparison between them could not be carried out. The average body temperature of the S strain was a little higher than that of the N strain, but this difference was also not significant. Furthermore we examined whether there are differences in body temperature between the resistant and susceptible breed or strain chicks during the first 8 days after hatching. Each chick's body temperature was recorded at the 0, 2, 4, 6 and 8th day after hatching. Fourteen White Leghorn and 13 New Hampshire chicks were used to determine the difference between breeds (Figure 2). The White Leghorn chicks increased their body temperature a little more quickly than did the New Hampshire chicks, but the differences in average body temperature during the first 8 days between them were not significant. The S strain and two sire families of the N strain, N_{187} and N_{526}, were used to determine strain difference of body temperatures. The numbers used were 17, 18 and 17, respectively. The average body temperatures of each groups are shown in Figure 3. The S strain chicks raised their temperatures more quickly than did the N strain chicks from the first day to the third day after hatching, but it might be due to the very low temperature of the S strain at the first day. During the first 8 days after hatching the differences in average body temperature among them were not significant.

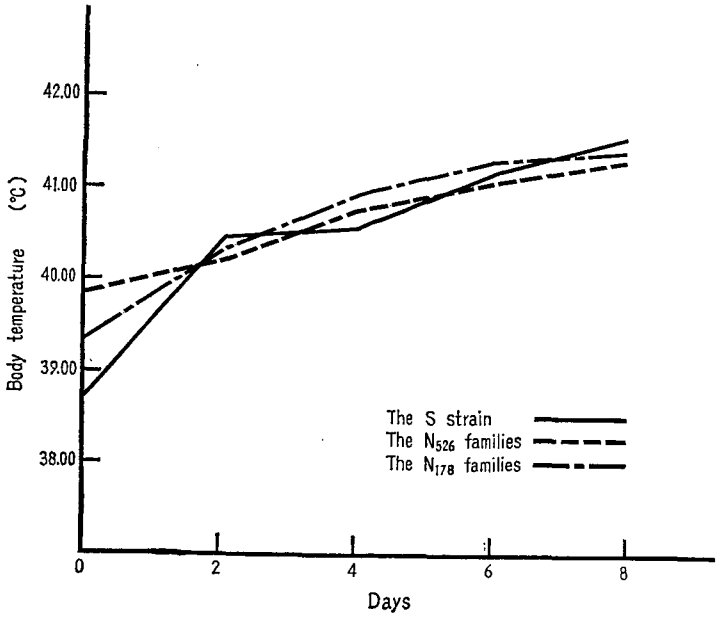

Fig. 3. Body temperature of the S strain and N_{178}, N_{526} sire families of the N strain from 0 to 8 days after hatching.

DISCUSSION

HUTT and SCHOLES (6) stated that there were breed differences in susceptibility to pullorum disease. The results obtained from the present study agree with above authors. In the present study, White Leghorns were more resistant than not only heavy breeds, but also Hinais and Onagas which are lighter than White Leghorns. The difference between White Leghorns and White Plymouth Rocks, however, was not significant. Hinais and Onagas are both Japanese native breeds and they showed high mortality. Onagas especially had the highest mortality.

JEFFRIES et al. (7) reported that there was a marked difference in susceptibility to pullorum disease between two of the test strains of White Leghorns. Differences among the strains of White Leghorns were also found in the present study. The S strain was more resistant than the N strain. We supposed that the virulence of *S. pullorum* varied from experiment to experiment on account of the fact that the mortalities were not always in proportion to the number of cells (Table 4). It seems plausible that the variation in virulence and numbers of cells was a cause of the differences among the replications.

ROBERTS and CARD (14) reported that offspring from the cross of the resistant strain with an unselected strain were fully as resistant as the resistant parents. The present data also showed the same result. This suggested that it was useful to establish a resistant strain first and to cross it with unselected strains next. There were no differences between reciprocal crosses. This indicated that maternal effects do not contribute to susceptibility to pullorum disease. According to ROBERTS and CARD (14), when the F_1 generation from the cross of the resistant strain with unselected strain was mated back to the resistant strain, the resistance of the offspring was somewhat lowered, but it remained high in comparision with that of chicks from the back cross of F_1 generation with an unselected strain. These differences could not be found in this study. This may be considered as due to the comparative smallness of the number of individuals used in the present experiment.

There is available no published paper dealing with relationships between blood groups and susceptibility to pathogenic organisms in chicks. Some research, however, has been reported in recent years on the fitness of the genotypes at the *B* locus. BRILES et al. (2), GILMOUR (4) and OKADA and MATSUMOTO (12) strongly suggested that some selection phenomenon favoring heterozygotes at the *B* locus is operating in chicken populations. And BRILES and ALLEN (1) reported that heterozygotes at the *B* locus were superior to homozygotes in both juvenile and adult viability. It seems that fitness, especially

viability, are affected by natural resistance to disease, no matter whether it is specific or nonspecific. In the present study, however, it was not obvious whether the genotypes at the *B* locus were related to susceptibility to pullorum disease or not. Further studies on this problem will be necessary.

Concerning the physiological basis for resistance to pullorum disease, there have been two different views. SEVERENS et al. (16) showed that chicks resistant to pullorum disease differred from susceptible ones in having a greater proportion of lymphocytes. On the other hand, SCHOLES and HUTT (15) reported that resistance was associated with superior control of the thermoregulatory mechanism. Attempts by RAM and HUTT (13) to discover any possible relationship between lymphocytes and susceptibility were unsuccessful and they supported the conclusion of SCHOLES and HUTT (15).

In the present study, the resistant strain showed significantly higher leucocyte numbers than that of the susceptible strain, but their F_1 generation which was as resistant as the resistant strain had lower numbers than did the resistant strain (Figure 1). From our data, no obvious relationship between total leucocyte number and resistance was found.

Although the difference of body temperatures among families was significant at the first day after hatching, they ceased being significant at the third day after hatching, (Table 12, 13). The average temperature of surviving chicks was a little higher than that of dead chicks though this difference was not significant (Table 14). And White Leghorns raised their temperature a little more quickly than New Hampshires (Figure 2). This suggested that if a large number of individuals were used for recording temperatures, there might be found some association between body temperature and resistance. In any case, it was not clear whether body temperature was related susceptibility to pullorum disease or not.

SUMMARY

Evidence presented here shows breed and strain differences in susceptibility to pullorum disease. White Leghorn chicks were more resistant than the other 4 breeds. The S strain chicks were more resistant than the N strain chicks in repeated tests of the strains of White Leghorns, and their F_1 generation chicks were fully as resistant as the S strain chicks. There were no significant differences between the reciprocal crosses of the S and N strains. The chicks from four different backcrosses of these F_1 generations to the S and N strains were as resistant as the S strain and F_1 generation chicks.

There was no difference between sexes in susceptibility. The relative susceptiblity of chicks classified by the *B* blood group locus genotypes was

examined. In the first experiment, the homozygous chicks were more resistant than the heterozygous chicks, but in the next experiment there were no difference between them.

During the first 15 days after hatching, the average leucocyte number of the S strain, which was the resistant one, was significantly higher not only than the susceptible N strain, but also than the F_1 generation which was as resistant as the S strain.

Familial differences in body temperature were significant at the first day after hatching, but not significant at the third day later. Surviving chicks after inoculation had a little higher temperatures one day after hatching than did dead chicks but this difference was not significant. White Leghorns raised their temperatures a little more quickly than did New Hampshires during the 4 days after hatching.

According to these data, resistance or susceptibility to pullorum disease could not be attributed to any single physiological character here studied.

ACKNOWLEDGMENT

We wish to thank Professor S. MIURA for providing the cultures of *S. pullorum*.

REFERENCES

1) BRILES, W. P. and C. P. ALLEN 1961. *Genetics* 46 : 1273.
2) ————, ———— and T. W. MILLEN 1957. *Genetics* 42 : 631.
3) DEVOLT, H. M., G. D. QUIGLEY and T. C. BYERLY 1941. *Poult. Sci.* 20 : 339.
4) GILMOUR, D. G. 1959. *Genetics* 44 : 14.
5) HUTT, F. B. and R. D. CRAWFORD 1960. *Can. J. Genet. Cytol.* 2 : 357.
6) ———— and J. C. SCHOLES 1941. *Poult. Sci.* 20 : 342.
7) JEFFRIES, C. D., D. E. HOLTMAN and J. A. CAMERON 1959. *Amer. J. Vet. Res.* 20 : 350.
8) LAMOREUX, W. F. and F. B. HUTT 1939. *Poult. Sci.* 18 : 70.
9) MATSUMOTO, K. and I. OKADA 1961. *Jap. J. Genet.* 36 : 257.
10) NATT, M. P. and C. A. HERRICK 1952. *Poult. Sci.* 31 : 735.
11) OKADA, I., S. TAKAGI and K. MATSUMOTO 1962. *Jap. J. Genet.* 37 : 102.
12) ———— and K. MATSUMOTO 1962. *Jap. J. Genet.* 37 : 267.
13) RAM, T. and F. B. HUTT 1955. *Amer. J. Vet. Res.* 16 : 437.
14) ROBERTS, E. and L. E. CARD 1935. *Illinois Agr. Exp. Sta. Bull.* 419 : 467.
15) SCHOLES, J. C. and F. B. HUTT 1942. *Cornell Univ. Agr. Exp. Sta. Memoir.* 244.
16) SEVERENS, J. M., E. ROBERTS and L. E. CARD 1944. *J. Infect. Disease* 75 : 33.
17) SNEDECOR, G. W. 1957. Statistical method. Iowa State College Press 5th edn. 316.