THE INHERITANCE OF DIFFERENCES IN THE LYSOZYME LEVEL OF HENS' EGG WHITE

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LYSOZYME is an enzyme first described by FLEMING (1922) which lyses many nonpathogenic bacteria by depolymerization of an acetylated amino-polysaccharide of their cell wall. Many of its chemical and physical attributes are now known; it is characterized by a relatively low molecular weight (about 15,000) and an unusually high isoelectric point (pH 10.8). Further details can be found in the excellent reviews by THOMPSON (1940) and HARTSELL (1949).

The occurrence of lysozyme is much wider than is generally realized. FLEMING's original paper described its presence in nearly all tissues and secretions of the human body. It is present in the tissues of most vertebrates and in some bacteria, but is absent in most plants tested. The highest concentration yet demonstrated is in the albumen of birds' eggs. FLEMING and ALLISON (1924), SMOLELIS and HARTSELL (1951), WETTER *et al.* (1953), and MACDONNELL *et al.* (1954) have reported large differences in lysozyme concentration in the albumens of different species of birds. The chicken ranked higher than all other tested species except the quail, whereas the duck and goose ranked among the lowest. Highly significant differences between strains of domestic fowl have been demonstrated both by COTTERILL and WINTER (1954) and by WILCOX (1956).

This report deals with the mating results of birds selected for differences in the lysozyme level of their egg white. The primary aim has been the elucidation of the genetic nature of such differences, but a secondary aim has been a clarification of the function of lysozyme in egg white. The amount of lysozyme found therein (4.5 mg/ml) is approximately 250 times as much as that required for maximal activity against susceptible bacteria; indeed, the enzyme is inactive at these high concentrations (WILCOX and DANIEL 1954). This seeming paradox suggests that lysozyme of egg white might have an additional function besides lysis of bacteria.

The procedure for investigation of these relationships has included the establishment and subsequent crossing of lines that differ genetically in respect to the lysozyme concentration of their egg white. An additional function of lysozyme might possibly be revealed by degree of association with differences in other traits in these lines. To offset the probable occurrence of chance differences unrelated to lysozyme concentration, this procedure was followed with two strains instead of one. Even so, it is realized that the occurrence of similar differences within both strains would provide little more than a hint, and that supporting evidence from additional sources would be required for proof of another function of lysozyme.

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MATERIAL AND METHODS

On the day after an egg was laid, its albumen was mixed for five seconds in a Waring blendor and stored in a corked shell vial at −20°C until analyzed for lysozyme. In no case did the period of storage prior to analysis exceed one month. Analysis for lysozyme was made by using the method of WILCOX and COLE (1954), which compares the amount of clearing of a suspension of bacteria by known amounts of egg white to that obtained by known amounts of crystalline lysozyme.

The general procedure followed was to analyze two dilutions of egg white from one egg laid when the bird was between 10 and 12 months of age. The basis for this procedure has been detailed elsewhere (WILCOX 1956). Deviations from the general procedure included: the use of only one dilution for each egg white in the survey of two strains; the analysis of two eggs laid two weeks apart for third generation birds; and an analysis of an additional egg from each of the second generation birds at seven months of age. All eggs from a given experiment or generation were always analyzed in a random sequence.

RESULTS

Variation within two strains

Although strains were known to differ in the lysozyme level of their egg white, it appeared that differences within a strain would lend themselves better to genetic analysis. An experiment was therefore performed for detection of exceptional families with respect to lysozyme level within two strains (C and K) of White Leghorn fowl maintained at Cornell University. Five birds were selected at random from each of 51 dam-families and an egg from each bird subsequently analyzed.

An analysis of variance of the results is given in table 1. Because of the disproportionate number of dam-families per sire, it was necessary to select five of them for use in the statistical analysis, but since this selection was at random the results can be extended to the survey as a whole. This analysis shows that there are highly significant differences due to dam, but that the differences between the six sires and between the two strains are not significant.

Selection of individuals for matings

After establishing that differences between dam-families exist it was a simple matter to select a high and a low family from each strain. Then, eggs from all hens in these

TABLE 1

Analysis of variance of data from a flock survey on the lysozyme concentration in egg white

Source	d.f.	Mean sq.	F.
Strain	1	2.53	5.58
Sire	4	0.453	0.35
Dam	24	1.305	2.08*
Within dam	120	0.626	

* Significant at the one percent level.

TABLE 2

Lysozyme concentration (mg/ml) in eggs of families from which individuals were selected for mating

	Families from which males were selected (flock survey data)			
	K High	K Low	C High	C Low
	5.7	3.3	3.5	4.8
	6.1	3.6	5.5	6.0
	4.3	3.9	5.2	4.3
	5.9	4.3	4.9	3.8
	5.0	4.9	6.2	4.2
Mean	5.40	4.00	5.06	4.62
	Families from which females were selected (flock survey data)			
	K High	K Low	C High	C Low
	5.3	3.6	4.6	3.7
	5.6	3.4	5.9	4.1
	5.5	3.7	5.7	4.2
	6.3	4.7	5.1	4.6
	6.5	3.9	6.5	3.9
Mean	5.84	3.86	5.56	4.10
Mean for entire family	5.55	4.39	5.24	4.40

families were subsequently tested to select extreme individuals to be used in test matings. The average for all females of the lowest family of K strain was much too high; consequently eggs from all females of the next lowest family were analyzed. The average for this family proved to be sufficiently low for use, and it was selected to be used as the low family of the K strain from this point on. Females from the four selected dam-families that were used for breeding had 5.6 and 5.2 mg lysozyme/ml egg white, or more, in the K High and C High families, respectively, and 3.8 and 4.3 mg/ml, or less, in the K Low and C Low families, respectively. Males were selected on the basis of the performance of five full sisters and were from different sire-families than the females to which they were mated. Four males were used in matings, one for each respective family of females. The families selected will henceforth be referred to as the first generation of the K High, K Low, C High, and C Low lines. Data used as a basis of selection of these lines are summarized in table 2.

The female progeny from these matings (second generation) were checked for lysozyme concentration by analysis of an egg laid at seven months of age. Birds with a lysozyme concentration of 5.8 mg/ml or more were selected in both of the High lines as parents for the third generation, whereas birds with 4.4 and 4.6 mg/ml or less were selected in the K Low and C Low lines, respectively. It should be noted that the lysozyme concentration in eggs laid at seven months of age is about 0.5 mg/ml greater than in eggs laid at ten months of age. Males were selected on the basis of an analysis of blood serum at eight weeks of age and on the performance of their sisters. A male from each line was mated to half sisters of his line and to first and second generation females in the opposite line of the same strain. In order to reduce inbreeding

as much as possible first generation dams as well as full sisters of selected males were used only in line crosses.

All birds within each generation were reared together. They were wing-badged before commencement of laying, and the badge number for a bird was assigned without regard to date of hatch, strain, or family. The different lines and crosses were never segregated, except for a special mating of 12 third generation K-strain females made during the seventh through ninth months of age.

Phenotype of the progenies

For a comparison of the lysozyme concentration of different lines and generations the data for eggs laid when the birds were between 10 and 12 months of age are considered. Most of the C strain data are not shown because of the great variation within both lines and the small numbers in the third generation. The differences between these lines were always small; the average lysozyme concentrations for the birds of the first, second, and third generations were 6.30, 4.88, and 5.39 mg/ml for the High line and 4.10, 4.39, and 4.49 mg/ml for the Low line.

Most of the discussion which follows will be concerned with the K strain. In this strain the average lysozyme concentrations for the birds of the first, second, and third generations were 5.96, 5.44, and 6.09 mg/ml for the High line and 3.60, 3.78, and 4.08 mg/ml for the Low line. The average for the K High × K Low cross was 4.65 mg/ml and for the K Low × K High cross was 4.73 mg/ml. Neither of these lines changed much in three generations. The slightly higher averages in the third generation may be an effect due to year or season, since birds of all the pure-line matings of both strains showed this increase. The average age at which the birds of this generation started to lay differed somewhat from that of the second generation, which was probably a consequence of the different dates of hatch (August and April for the second and third generations, respectively). This might have influenced the lysozyme

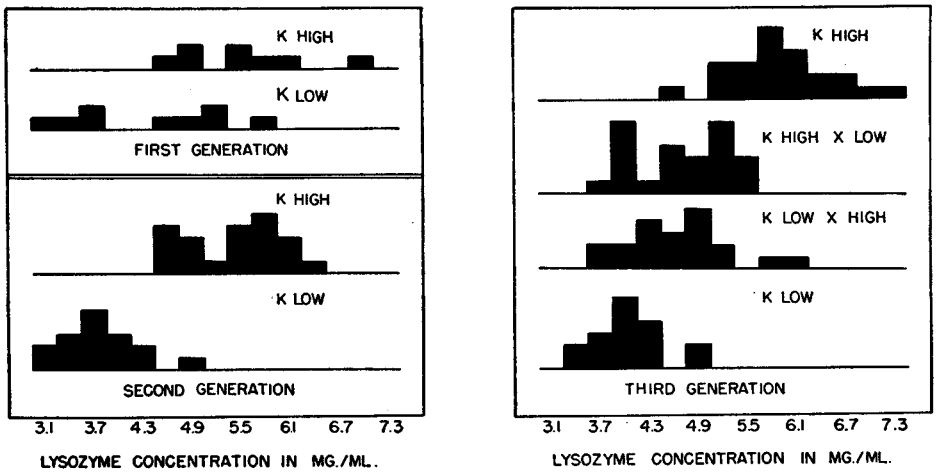

FIGURE 1.—Histograms of the lysozyme concentration of the egg whites of first, second, and third generation birds of the K strain.

TABLE 3

Performance of the High and Low lines in respect to other characteristics

Characteristic	K High Generation		K Low Generation		C High Generation		C Low Generation	
	2nd	3rd	2nd	3rd	2nd	3rd	2nd	3rd
No. of birds whose eggs were analyzed for lysozyme	21	23	16	17	18	9	17	11
No. eggs 9 through 11 months of age (5-day trap period)[a]	42	46	41	39	43	46	43	48
Age at first egg (days)[a]	181	200	187	227	173	196	177	218
Mortality to 210 days of age (%)	0	14	8	10	12	21	4	14
Mortality from 210 days of age to 12 months of age (%)	11	11	23	8	0	0	12	0
Body weight at 11 months of age (grams)[a]	—	1973	—	1878	—	2042	—	2280
Egg weight at 10 months of age (grams)[a]	58	59	55	56	56	56	55	57
Albumen quality (Haugh units)[a]	94	95	85	83	72	76	78	79
pH of egg white[a]	8.38	—	8.52	—	8.43	—	8.46	—
Total solids of egg white (mg/ml)[b]	—	129	—	121	—	131	—	128
Total normality of K and Na of egg white[c]	0.110	—	0.116	—	0.113	—	0.112	—

[a] Average of birds whose eggs were analyzed for lysozyme at ten months of age.

[b] Average of four eggs per line.

[c] Average of five eggs per line.

concentration at ten months of age. The other striking change was the decrease in lysozyme concentration in the second generation of the K High line. Histograms of the lysozyme concentration for all three generations of the K strain are presented in figure 1, where these data have been arranged in 0.3 mg/ml groupings.

Data collected to detect any relationship between lysozyme level and other traits are shown in table 3. There appears to be little relationship between differences in lysozyme concentration and the number of eggs laid in a three month period, mortality, body weight, or total normality of K and Na (which are activators of lysozyme). Differences in age at production of first egg are probably not related to lysozyme since there was no regression within lines of lysozyme concentration on this characteristic. Eggs from the K High line weighed more than those from the K Low line, a relationship which has been observed before (WILCOX 1956). A positive regression of albumen quality on lysozyme concentration has been shown (WILCOX 1955), and is evident in the K strain but not in the C strain. There may be a slight association of lysozyme concentration with total solids as well as with pH of egg white, but there is no further evidence bearing on this.

Genetic considerations

The establishment within two strains of high and low lines in respect to the lysozyme level of egg white provides evidence of genetic differences for this characteristic.

An influence of the dam on the lysozyme level of egg white in eggs laid by her progeny is provided in the pure lines and crosses of the third generation, where the same K High male when mated to both K High and K Low females produced correspondingly different progenies. A similar influence of the female was manifest in

TABLE 4

A comparison of daughters from the same dam which had been mated to a High and a Low line male

Line	Lysozyme concentration in mg/ml		
	Dam	Mean for daughters mated to male of	
		Same line	Opposite line
K High	5.6	5.85 (4)	4.34 (7)
	6.9	5.42 (5)	4.25 (2)
K Low	3.7	3.52 (6)	4.98 (5)
	3.3	3.80 (6)	4.88 (4)
C High	6.7	4.93 (7)	4.78 (8)
	6.3	5.10 (3)	4.97 (7)
C Low	4.3	4.46 (7)	4.58 (5)
	3.8	4.51 (7)	4.47 (3)

( ) Indicates number of daughters on which mean is based.

matings involving the same K Low male to both types of females. Data from crosses of the C High and Low lines showed a similar trend.

An influence of the sire on the lysozyme level of the egg white in eggs laid by his progeny is illustrated in table 4, which shows the performance of progeny of first generation females that had been mated to males of their line to produce the second generation, and then to males of the opposite line to produce crosses in the third generation. It is evident that there is a large influence of the male on the progeny from each female in the K strain, but there is apparently much less of this influence in the C strain. The poor success in breeding for differences in lysozyme level in the C strain may be due either to use of genetically similar males or to an insignificant influence of the sire on the lysozyme level of his daughters' eggs. The former seems more likely to be true since dams of the C strain tended to produce daughters whose average lysozyme level was closer to the strain average than were their own eggs, a tendency which was very much reduced in the K strain.

Although there were large differences between the mean lysozyme level of the egg white of the K High and Low lines, there was a considerable variation within each line. Certain observations suggested that this variation might be nongenetic in nature. These were the small change in the lysozyme level of the two lines in three generations, suggesting homozygosity, and the similar performance of progenies from dams of the same line with differing phenotypes.

To determine whether or not this within line variation was genetic in nature, females of the third generation with very high and very low concentrations of lysozyme in both the K High and K Low lines were mated to the same (K High) male. The results of these matings are shown in table 5. Unfortunately the number of females obtained from certain of these matings was small. The females from both the lower end of the K Low line and the upper end of the K High line produced eggs which were much closer to the mean lysozyme concentration of the flock than were those obtained in the second and third generations. There does nevertheless seem to be some difference within both the K High and K Low lines in the ability of the females to pass on to their progeny high or low lysozyme concentration. It is noteworthy that those

TABLE 5

Results of mating the same K High line male to females of the K High and K Low lines with an unusually high or low lysozyme level for their line

Line	Position of dam's phenotype in histogram for third generation	Lysozyme concentration in mg/ml	
		Dam	Mean for daughters[a]
K High	Upper End	7.4	5.47 (3)
		6.3	5.30 (1)
		6.3	4.90 (1)
	Mean	6.67	5.32 (5)
	Lower End	5.3	5.13 (6)
		5.2	5.25 (2)
		4.7	5.19 (8)
	Mean	5.08	5.18 (16)
K Low	Upper End	4.9	5.00 (4)
		4.4	5.44 (8)
		5.0	4.75 (4)
	Mean	4.77	5.16 (16)
	Lower End	3.6	4.27 (3)
		3.7	4.93 (4)
		3.3	5.10 (1)
	Mean	3.53	4.70 (8)

[a] Followed by number of daughters on which mean is based.

dams of the two lines that were nearly identical phenotypically produced daughters nearly identical in the lysozyme concentration of their eggs. Matings of a K Low male of the third generation to similar females produced four daughters with an average lysozyme level of 4.08 mg/ml.

A number of gene pairs probably effect the lysozyme level of egg white, as indicated by the presence of genetic differences within two lines which themselves differ in their average lysozyme level. The inheritance is apparently nuclear and autosomal since there was no difference between reciprocal crosses.

Mating results suggest partial dominance of low lysozyme concentration. This relationship may be spurious, since the K Low females started to lay nearly 30 days later than females of the K High line and the reciprocal crosses. This difference in time may have influenced the lysozyme concentration of eggs laid at ten months of age. Evidence in favor of dominance of low lysozyme concentration versus an additive effect is therefore somewhat tenuous.

DISCUSSION

The reported situation is basically different from that usually encountered in genetic studies involving an enzyme, in which the presence or absence of a normally

functioning enzyme is governed by a single pair of alleles. The genetic differences obtained in lysozyme concentration might well be the result of modifying genes affecting synthesis or secretion of the enzyme, or the presence of additional genes for lysozyme. It has been found that these genetic differences are evidently not associated with changes in utilization of vitamin A or tryptophan. Knowledge of what other substances are increased to give a greater weight of total solids in the egg white of High lines might be enlightening.

The significance of the genetic differences in lysozyme concentration of egg white is not fully understood. The only definite associations with other characteristics that have been shown to date are the positive regression of both egg weight and albumen quality on lysozyme concentration. Thus on the basis of present knowledge on the subject, hens with high levels of lysozyme in their egg white have an advantage from the standpoint of artificial selection in respect to these characteristics. One would expect that any selection for high egg weight or albumen quality will favor hens with higher amounts of lysozyme in their eggs, but actually only a minority of the eggs analyzed in the flock survey were high in their lysozyme level. Two possible explanations for lack of genetic fixation are heterosis and random distribution. Heterosis could be the underlying factor if there were an advantage of low lysozyme concentration for the expression of some other trait or traits, whereby the heterozygote might be the best compromise from the standpoint of all associated traits. Such an advantage of low lysozyme concentration has yet to be demonstrated. Random variation remains a possibility; there has been little selection for albumen quality in the stock studied, but there has been some for egg weight.

This study provides a rather interesting case of genetic variation in concentration of an enzyme. However, one should be cautious in using these reported results to draw inferences concerning other enzymes. Lysozyme is not a typical enzyme in respect to its stability, molecular weight, and isoelectric point, and particularly in respect to its extremely high concentration in egg white.

SUMMARY

Eggs from a large number of families of two different strains of White Leghorns were analyzed for lysozyme. Two families from each strain were selected for high or low lysozyme concentration in their egg white, and exceptional hens from each were mated with males from comparable families. The eggs from their daughters and granddaughters were subsequently analyzed for lysozyme.

In the K strain little change in the lysozyme concentration of the egg white was found following the initial selection in both High and Low lines, whereas in the C strain a substantial regression towards the flock mean resulted. The lysozyme levels of reciprocal crosses were intermediate to those of the parental lines.

It has been concluded that in the K strain both parents exerted a substantial influence on the lysozyme concentration shown by their daughters through action of autosomal genes. The available evidence has suggested a number of gene pairs controlling the level of this enzyme in albumen, probably without dominance. In the C strain there was some influence of the dam but very little of the sire.

Lines differing greatly in lysozyme concentration of egg white did not differ to any extent for several other traits, except for a tendency for eggs high in lysozyme to

weigh more and to possess firmer albumen. The biological significance of the genetic differences in lysozyme concentration and the reason for genetic heterogeneity in the stock studied remains unclear.

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