Parthenogenesis in Eggs of White Leghorn Chickens Following an Outbreak of Visceral Lymphomatosis. (31304)

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An experiment was planned at Beltsville in 1960 to determine if strains of White Leghorns (WL) chickens differed in their ability to withstand high summer temperatures. Chicks for the proposed tests came from two sources: one group of 100 females was hatched from non-pedigreed eggs obtained from another station; the second group of 100 females from eggs laid by an outbred, mass-mated flock of birds at this station. Both groups of chicks were hatched concurrently in the same forced-draft incubator.

A severe disease outbreak, diagnosed as visceral lymphomatosis (VL), occurred in each of the 2 groups when the chicks were only 5-6 weeks of age.* Heavy mortality continued throughout the remainder of the brooding and growing period. Only 37 of the original 200 females were alive on March 1, 1961.

Tests were carried out during the spring of 1961 to see if this severe viral infection had induced any change in the potentiality for parthenogenesis, notably low in eggs of Beltsville White Leghorns(1). It was found that 4 of the hens, following the infection, were producing a significant number of unfertilized eggs which underwent development upon being incubated. This finding led to the decision to make a series of matings to determine if this seemingly virus-associated trait could be transmitted to progeny. A second objective was to determine if types of sires would have any significant influence on the incidence of parthenogenesis in eggs of their daughters.

Materials and methods. In testing for parthenogenetic potential, unfertilized eggs of progeny were collected daily over a period of 60 to 90 days. Each egg, as collected, was identified as to hen number and date of lay. Daily collections of eggs were placed in an incubator each evening and incubated for a period of 8-10 days at a temperature of 99.5°F and a relative humidity of 57%. Following this period of incubation, eggs were removed, broken individually, and examined for parthenogenetic development. Eggs with discs which had attained a diameter of 6 mm or more were classified as having undergone parthenogenetic development.

Each year, following completion of parthenogenetic tests, a certain number of hens were mated to obtain stock for the following season. The mating scheme is outlined in Table I. Observations on survivors of the viral outbreak demonstrated that eggs of hen 9243 exhibited the highest incidence (37.5%) of parthenogenesis. During the 1961 breeding season this hen was mated to a Dark Cornish male. Of the 8 daughters producing parthenogenetic eggs, 6 served as breeders in the spring of 1962. These 6 hens were inseminated with pooled semen from 2 Dark Cornish males. After a sufficient number of fertile eggs had been obtained, fertility was allowed to run out, whereupon the 6 hens were reinseminated with pooled semen from 2 White Leghorn males.

Starting in 1963 a limited number of young, virgin, crossbred females (hens whose sires were Dark Cornish and whose dam's pedigree traced to hen 9243) were mated. Only females which as virgins had produced eggs showing a high incidence of parthenogenesis served as breeders. Approximately equal numbers of selected hens were placed in each of two groups. Hens of one group were inseminated with semen (pooled samples) contributed by a number of Dark Cornish males; the other hens with semen from White Leghorn males. After a sufficient num-

* The possibility of a separate viral infection cannot be excluded. The very early mortality occuring in the original White Leghorn stock (5-6 weeks) is suggestive of Marek's disease; that occurring in subsequent generations (2-3 months) after sexual maturity is typical of lymphoid leukosis.

TABLE I. Incidence of Parthenogenesis Found in Eggs of White Leghorn Hen 9243, a Survivor of a Severe Outbreak of Visceral Lymphomatosis. Also shown are percentages of parthenogenesis encountered in eggs of descendants of hen 9243 and in those of Dark Cornish.

Breeding season	Group	Parental stock: Sires No.	Sires Breed	Dams No.	Dams Breed or cross	Progeny: Daughters No.	Daughters Breed or cross	No. of eggs	% Parthenogenetic development
1960	Exp	—	WL	—	WL	1(9243)	WL	24	37.5
	Control	—	DC	—	DC	26	DC	341	5.0
1961	Exp	1	DC	1(9243)	WL	8	G_1	525	32.8
	Control	1	DC	6	DC	22	DC	795	7.2
1962	Exp	2	WL	6	G_1*	13	G_2	475	.2
	Exp	2	DC	6	G_1	39	G_2	1201	11.2
	Control	3	DC	6	DC	13	DC	437	5.9
1963	Exp	20	WL	27	G_2	69	G_3	3423	.7
	Exp	5	DC	27	G_2	42	G_3	2161	15.0
	Control	4	DC	7	DC	15	DC	435	5.8
1964	Exp	15	WL	24	G_3	86	G_4	3181	2.0
	Exp	15	DC	24	G_3	82	G_4	2103	14.1
	Control	5	DC	11	DC	22	DC	625	18.9
1965	Exp	19	WL	27	G_4	87	G_5	3171	.56
	Exp	9	DC	27	G_4	61	G_5	1267	19.7
	Control	7	DC	12	DC	31	DC	706	17.2

* Subscripts indicate number of generations descendants are removed from hen 9243.

ber of fertile eggs had been obtained, fertility was allowed to run out. Hens of both groups were thereupon reinseminated; those previously inseminated with Dark Cornish semen were reinseminated with semen of White Leghorn males, while hens of the second group were reinseminated with semen from Dark Cornish males. Groups of purebred Dark Cornish females (sired by one or more of the Dark Cornish males used in other matings) served as controls. The number of males used in matings each year, together with the number of females with which they were mated, are given in Table I.

All Dark Cornish males were of Beltsville origin, representing a strain of birds in which considerable selection had been practiced toward a high incidence of parthenogenesis. The White Leghorn males were likewise of Beltsville origin, having come from healthy flocks with a history of a very low incidence of parthenogenesis(1).

Chicks were brooded and raised in isolated quarters in an attempt to keep them free from disease. Use of live virus vaccine was intentionally avoided so as not to influence the level of parthenogenesis(2,3).

At maturity, the virgin females were transferred to individual wire cages in a heated building. Hens were given a standard all-mash ration and maintained under a 14-hour light day. Birds which died were autopsied and data were collected on causes of death.

Results and discussion. During the period, February 1 to March 12, 1961, a total of 1,053 eggs were laid by the 37 purebred White Leghorns which survived the outbreak of visceral lymphomatosis. Six hundred and forty-eight of these eggs were laid by the 20 hens of outside origin and 405 by the 17 hens of Beltsville origin. No evidence of parthenogenesis could be detected in the 648 eggs laid by imported stocks. On the other hand, 17 eggs (4.2%) were encountered among the 405 eggs laid by the 17 surviving hens of Beltsville origin. All eggs exhibiting development were laid by 4 hens; the average incidence of parthenogenesis in eggs of these 4 hens was 15.1%.

Eggs produced by White Leghorn hen 9243, as noted earlier, showed the highest incidence (37.5%) of parthenogenesis. Each of her 8 daughters sired by a Dark Cornish male produced some eggs which underwent a limited, unorganized type of parthenogenetic development, varying from 13.7 to 57%.

The average incidence of parthenogenesis in eggs of the 8 daughters of hen 9243 was

32.7%, a value only slightly lower than that encountered in unfertilized eggs of their dam. On the basis of previous results(1), one would have expected this value to have been somewhat closer to the average figure shown by purebred Cornish. The reverse situation, however, was found to exist in the recorded level of parthenogenesis among eggs of the second generation (Table I). In this instance only 11.2% parthenogenesis was encountered, an average value much closer to that shown by Dark Cornish than their crossbred dams. These average percentage values, while seemingly departures from the expected, are not surprising in view of the fact that only one Dark Cornish male was involved in matings in 1961 and only 2 in 1962. In earlier studies involving turkeys it was found that much variation exists among individual sires, as well as dams of parthenogenetic strains, in their ability to produce offspring having a marked predisposition for parthenogenesis (unpublished observation).

Percentages of parthogenesis encountered in eggs of virgin hens representing the 3rd, 4th, and 5th generation removed from White Leghorn hen 9243 are also given in Table I. Percentage values for eggs of Dark Cornish serving as controls are also given. It should be pointed out that the average percentage values given in Table I for parthenogenesis in eggs of Dark Cornish in 1964 and 1965 are more than 2 to 3 times higher than in previous years. Moreover, the highest incidence of parthenogenesis in eggs of females sired by White Leghorn males (2.0%) was also encountered in 1964. No explanation can be offered for these high values.† The average percentage incidence of parthenogenesis in eggs of hens sired by White Leghorn males varied from 0.2% in 1962 to 2.0% in 1964. These values are only a fraction of those recorded in each respective year for eggs of females sired by Dark Cornish males.

Considerable mortality from visceral lymphomatosis occurred each year among hens whose pedigree traced to White Leghorn hen 9243. Practically all deaths occurred after pullets had been placed in laying cages, usually starting 2-3 months after onset of egg production. Mortality records are complete for 1964 and may be considered typical of losses sustained in other years. Thirteen of the 168 birds whose pedigree traced to 9243 (4th generation) died during the 1964 season. Eleven birds on autopsy were found to have had visceral lymphomatosis. Of the 11 birds, 9 were sired by Dark Cornish and 2 by White Leghorn males. No mortality occurred during the same period among the 22 Dark Cornish controls.

A number of older hens of the parthenogenetic strain each year have developed large ovarian tumors which have been tentatively identified by Dr. Robert Furrow, pathologist, Inspection Branch, Poultry Division, Consumer and Marketing Service, at this station, as granulosa-cell tumors. Some of these tumors attained weights of 200-300 g and were readily detectable in living birds by digital palpation. Seven of the 8 daughters of hen 9243 developed such tumors. Two of these 7 daughters died the latter part of their first laying season; the others during their second and third years of production.

Parthenogenesis is rarely encountered in unfertilized eggs of most healthy flocks of domestic chickens, *i.e.*, to the extent that the embryonic tissue reaches a stage of growth during incubation that is visible macroscopically(4). More than 35,000 unfertilized chicken eggs produced by 8 representative breeds have been incubated and examined for parthenogenetic development at Beltsville. Only eggs of Dark Cornish or crosses involving Dark Cornish showed a significant incidence of parthenogenetic development. This recent study included eggs of both outbred and inbred Single Comb White Leghorn chickens of Beltsville origin(1). Of the 2,507 White Leghorn eggs tested, only one showed development. This finding is especially significant in view of results of the present investigation, where 4 of 17 White Leghorn hens of Beltsville origin, following a severe, naturally occurring epizootic of visceral lym-

† Through pedigree error, one of the 5 Dark Cornish males selected as a breeder in 1964 was not 100% pure Cornish. Incorporation of this male's blood line may have raised slightly the average level of parthenogenesis in eggs of daughters and granddaughters.

phomatosis, produced eggs averaging 15% parthenogenesis.

The results of this investigation offer at least circumstantial evidence for the possible existence of some association between an infection with a leukosis virus and parthenogenetic development. However, it must be recognized that the genetic changes resulting in a high incidence of parthenogenesis in the Beltsville White Leghorn stocks may have had their origin in some source not related to the viral outbreak. The nature of the physiological mechanism through which the virus may have operated to produce this effect is not clear. It is certain, however, that not all birds responded in exactly the same way, since only 4 of the 37 survivors gave evidence of having acquired this predisposition toward parthenogenesis.

It is clear that some changes must have occurred in the germ cells of hen 9243, and presumably, in the case of the other 3 White Leghorn hens, although these birds were not involved in matings. This change, once induced, appeared to be at least semipermanent, since in the case of hen 9243, the predisposition to parthenogenesis was passed on to each of her 8 daughters, and it has persisted in this line of birds to the 5th generation.

Data presented in Table I also make clear that the genotype of the sire has a marked influence on the expression of parthenogenesis in eggs of his offspring. Sires from lines of birds with a history of low incidence of parthenogenesis (WL), produce progeny whose eggs show little predisposition to develop parthenogenetically. This is true even though the selected females to which they were mated, as virgins, demonstrated their ability to produce eggs showing a high incidence of parthenogenetic development. On the other hand, sires from stocks which have been selected for parthenogenesis (Dark Cornish of Beltsville breeding) on being mated to females having a predisposition to parthenogenesis, give rise to daughters, a substantial number of which showed a marked tendency toward parthenogenesis.

Summary. Four Single Comb White Leghorn hens of a nonparthenogenetic strain, following a severe outbreak of visceral lymphomatosis, produced a significant number of unfertilized eggs, which upon being incubated, underwent parthenogenetic development. One hen (9243) produced unfertilized eggs, 37.5% of which exhibited parthenogenesis. This hen was subsequently mated to a Dark Cornish male from a strain averaging 5-7% parthenogenesis. Each of 8 virgin daughters of hen 9243 produced some eggs which underwent parthenogenetic development. Eggs of individual hens varied from 13 to 57%, while the average for the entire group was 32.7.

Daughters of hen 9243 were subsequently mated first to Dark Cornish males and later to White Leghorn males of Beltsville breeding (non-parthenogenetic strain). Tests for parthenogenesis involving eggs of progeny revealed that the predisposition to parthenogenesis was transmitted from parents to progeny. Use of White Leghorn sires, however, tended to suppress parthenogenesis in eggs of their virgin daughters, while Dark Cornish males allowed a fuller expression of this trait.

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